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LOCAL TISSUE REACTIVITY (SHWARTZMAN PHENOMENON) IN THE HEART AND THE FEMORAL ARTERY OF THE RABBIT

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IN THE production of the phenomenon of local tissue reactivity (Shwartzman [1]) a new category of toxic antigens has been demonstrated in bacterial "agar washing" filtrates obtained from a variety of pathogenic micro-organisms. From the standpoint of physiochemical properties these antigens are similar to the usual bacterial exotoxins; yet the tissue response which they elicit in the experimental animal differs from that observed after the introduction of bacterial exotoxins prepared in the usual way. The usual toxins inflict damage in direct proportion to their strength; this is true also for the bacterial "agar washing" filtrates, but in addition it has been shown that when a local injection of a subtoxic dose of filtrate is followed twenty-four hours later by an intravenous injection of the same filtrate diluted to such a point as to be unable per se to produce any remarkable tissue reaction, a striking necrotic-hemorrhagic inflammatory change is produced at the site of the local injection.

This course of events, first described in the skin,[2] has been uniformly produced in many parts of the body: lungs,[3] stomach,[4] knee joints,[5] appendix,[6] adrenal glands,[7] conjunctivas,[8] pancreas,[9] kidneys [10] and

From the Mount Sinai Hospital Laboratories (Dazian Fellow), New York.

1. Shwartzman, G.: The Phenomenon of Local Tissue Reactivity, New York, Paul B. Hoeber, Inc., 1937.
2. Shwartzman, G.: J. Exper. Med. **48**:247, 1928.
3. Shwartzman, G.: J. Exper. Med. **51**:571, 1930.
4. Karsner, H. T.; Ecker, E. E., and Jackson, E. L.: Proc. Soc. Exper. Biol. & Med. **29**:319, 1931.
5. Moritz, A. R., and Morley, J. D.: Proc. Soc. Exper. Biol. & Med. **29**: 321, 1931.
6. Latteri, S.: Riv. di pat. sper. **13**:389, 1934.
7. Gronchi, V.: Boll. Soc. ital. di biol. sper. **9**:1032, 1934.
8. Cassuto, N.: Sperimentale, Arch. di biol. **87**:191, 1933.
9. Reitano, R., and Loi, L.: Boll. Soc. ital. di biol. sper. **9**:196, 1934.
10. Shwartzman, G.; Bernheim, A. I., and Gerber, I. E.: J. Mt. Sinai Hosp. **4**:1020, 1938.

neoplastic tissue.[11] In all these experiments, regardless of the site and of the method of preparation, the development of the phenomenon was consistently characterized by a severe vascular response with hemorrhages, extreme venous dilatation and engorgement, and thrombosis of venules and capillaries. Since an explanation of this peculiar manifestation could not be found on purely morphologic ground, it was thought that a condition of endothelial hypersensitivity initiated by the local preparatory injection and activated by the reacting factors reaching the vessels by way of the blood stream accounted both for the vascular damage and for the hemorrhagic tissular change.

The present investigation is mainly concerned with the phenomenon as observed histologically in the heart and the femoral artery. Since the procedures involved in these experiments established conditions that might be compared in certain aspects with those studied by others in artificially produced allergic states and with carditis and angiitis induced by bacterial toxins, a comparative analysis will be made and integrated with some further observations in which the effect on the heart and a peripheral artery of a non-phenomenon-producing substance was studied.

MATERIAL AND METHODS

The experiments were divided into the following two main groups: (*a*) the production of the phenomenon in the heart and a femoral artery by the use of a bacterial filtrate, and (*b*) duplicate experiments in which rheumatic blood was used. In the first group of experiments these procedures were followed:

1. A single intracardiac injection of bacterial filtrate (18 rabbits).
2. A single intravenous injection of the filtrate (8 rabbits).
3. An intracardiac injection followed by an intravenous injection (16 rabbits)—the cardiac Shwartzman phenomenon.
4. A local periarterial and an intra-arterial injection (9 rabbits).
5. A local periarterial injection followed by an intravenous injection (3 rabbits)—the periarterial Shwartzman phenomenon.
6. A local intra-arterial injection followed by an intravenous injection (3 rabbits)—the intra-arterial Shwartzman phenomenon.
7. A combined arterial and cardiac phenomenon (4 rabbits).

Stock rabbits of an average weight of 2 Kg. were used as experimental animals. "Agar washing" filtrates of a twenty-four hour old culture of meningococcus (type B, group 3) prepared according to the method described by Shwartzman were employed both for the local preparatory injection and for the provocative intravenous injection.

For the intracardiac preparation the filtrate was injected directly into the heart through a 5/8 inch (1.5 cm.), 25 gage hypodermic needle inserted in a depilated area of intact thoracic wall. The effect of the trauma by itself was determined by a control procedure in 4 animals which were given local and intravenous injections, singly and combined, of saline solution. No evidence of damage was encountered in any of these.

For the arterial preparation the femoral artery was exposed under aseptic technic and the filtrate injected by hypodermic needle either into the periadventitial tissue or directly into the lumen of a double ligated tract of the vessel.

11. Gratia, A., and Linz, R.: Compt. rend. Soc. de biol. **108**:427, 1931.

The traumatic effect of the surgical maneuver necessary to prepare the femoral artery and to close the wound was determined in each animal individually by exposing and closing the same vessel of the other extremity and later examining the site both grossly and microscopically.

In all animals the provocative intravenous injection of the filtrate was given in the marginal vein of an ear twenty-four hours after the preparatory injection.

In order to compare the effects of a phenomenon-producing substance (bacterial filtrate) with those elicited by a non-phenomenon-producing substance, the same experimental procedures were repeated in another series of animals with blood from a patient who had rheumatic fever. As in the experimental elicitation of the phenomenon, the necessary control experiments were carried out.

1. A single intracardiac injection of rheumatic blood (2 rabbits).
2. A single injection and double (one week apart) intravenous injections of rheumatic blood (6 rabbits).
3. An intracardiac injection followed by an intravenous injection (6 rabbits).
4. A local intra-arterial injection (3 rabbits).
5. A local intra-arterial injection followed by intravenous injection (3 rabbits).

The intracardiac injection was performed according to the procedures already described, 0.5 cc. of blood being used; 2 cc. was used for the intravenous injection, and for the local arterial preparation several drops of blood was injected into the lumen of a ligated section of a femoral artery. The animals which had received the single intravenous injection, the double intravenous injections and the single intracardiac injection were killed after twenty-four hours. The animals which had received the intracardiac injection followed by an intravenous injection twenty-four hours later were killed, 2 at twenty-four hours, 2 at the third day and 2 at the seventh day. The animals given the single local intra-arterial injection and the local intra-arterial injection followed by an intravenous injection twenty-four hours later were killed after one day.

The patient from whom the blood was obtained had had a recent and severe attack of rheumatic fever with cardiac involvement and generalized swelling of the large joints. When the samples of blood were taken, the disease process appeared to be in remission, and the temperature was normal. Repeated blood cultures were constantly negative. Control experiments were carried on in a few rabbits, blood from normal persons being used. The impression obtained was that the microscopic findings were somewhat different from those noted in the rabbits inoculated with the blood of the rheumatic patient; however, since this point will be investigated further, no conclusions are made at present.

THE CARDIAC AND ARTERIAL SHWARTZMAN PHENOMENON

1. Effect of a Single Intracardiac Injection of Bacterial Filtrate.

In order to obtain a dilution of filtrate of such minimal strength as to be unable to elicit any remarkable tissue response on being injected into the myocardium once, increasing dilutions of filtrate were given in a fixed amount (0.3 cc.) to a series of 18 rabbits. Four animals received undiluted filtrate and were killed at the twenty-fourth hour. Fibrinous pericarditis and exudative myocarditis, with polymorphonuclear leukocytes infiltrating the involved tissues to a striking degree, were consistent findings in all 4, and in 1 a firmly adherent mural thrombus was found implanted on the endocardium lining the left ventricle. Similar changes were shown by 3 other animals which had received dialyzed and frozen filtrate diluted 1:3. The same filtrate at the dilution of 1:20 was found to be productive

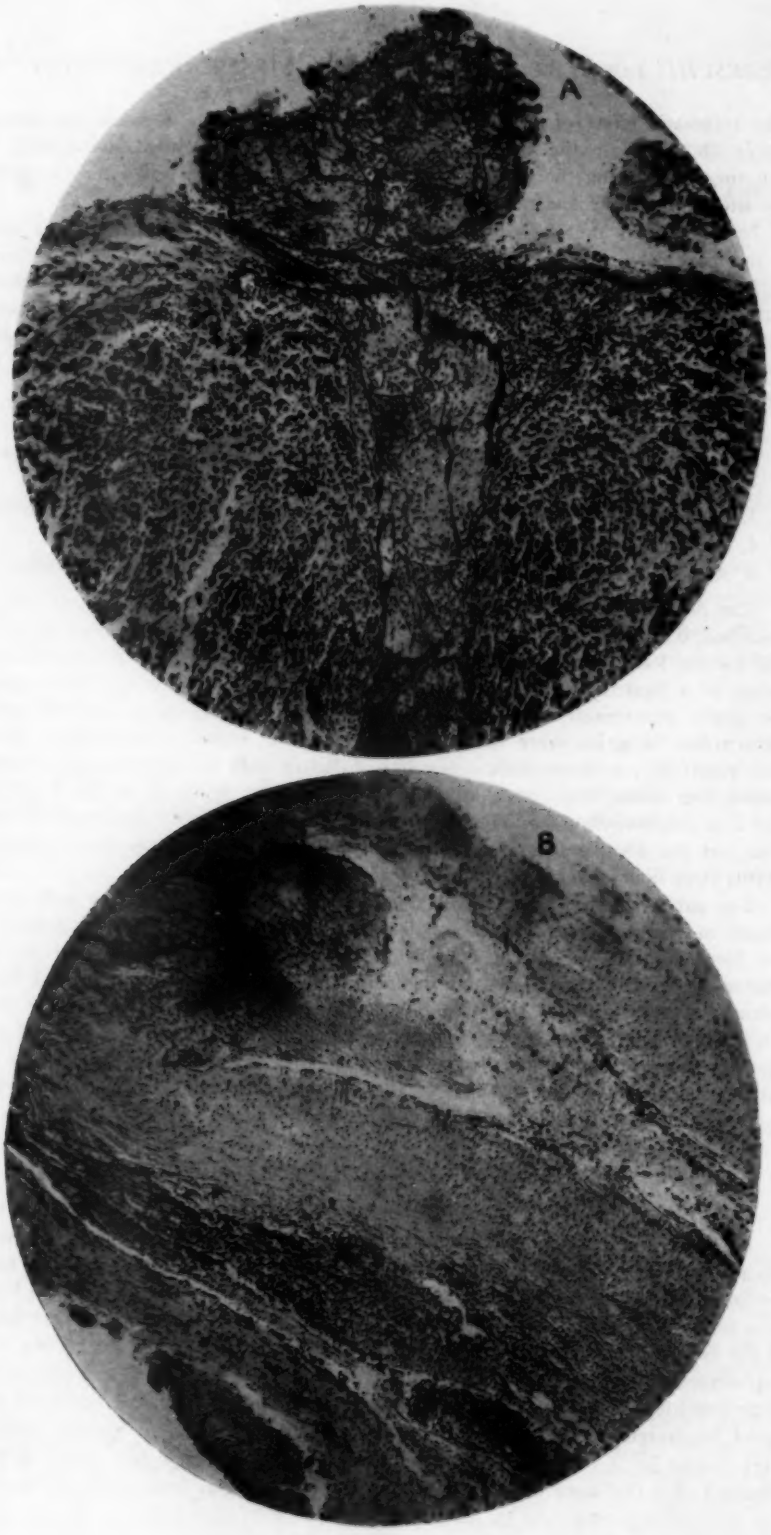

Figure 1
(See legend on opposite page)

of a much milder reaction in 2 other animals, and an inconstant negligible tissue response followed the injection of dialyzed filtrate diluted 1:30 in 9 rabbits. The 1:30 dilution of the filtrate was therefore considered satisfactory as the preparatory dose and was used as such in the production of the phenomenon.

2. Effect of a Single Intravenous Injection of the Filtrate.

The effect of a single intravenous injection of bacterial "agar washing" filtrate on the heart as well as on the other organs has been analyzed by Gerber.[12] A few trials enabled us to confirm his observations (figs. 2*B* and 3*A*). Four rabbits were given an intravenous injection of 2 cc. of filtrate diluted 1:10. In these animals the only evidence of cardiac damage was a waxy appearance in groups of myofibrils and a moderate interstitial inflammatory reaction, mostly on the part of polymorphonuclear leukocytes. In 4 other rabbits given an injection of the same filtrate dialyzed and diluted 1:20, no evidence of myocardial damage was found, and this dilution was therefore thought satisfactory for the provocative injection.

3. Effect of the Intracardiac Injection Followed by an Intravenous Injection.

Five rabbits received plain bacterial "agar washing" filtrate; 11 received dialyzed and frozen bacterial "agar washing" filtrate. Regardless of the material used, all animals showed an identical pattern of lesions.

Gross Findings.—In 10 animals killed twenty-four hours after the provocative intravenous injection, layers of a flaky fibrinous exudate were found on both the epicardial and the pericardial membrane. There were concomitant hemorrhagic changes, either limited to the site of the local preparatory injection or massive in distribution and involving the entire pericardium in a mottled fashion. The epicardial vessels were visibly engorged, and petechial hemorrhages outlined their course.

The underlying myocardium presented a markedly variegated appearance owing to alternating yellowish gray, dry, friable areas grossly suggesting necrobiotic change and reddish brown hemorrhagic areas involving the entire thickness of the myocardium to the parietal endocardium. In 3 of these 10 animals, large thrombotic masses firmly adherent to the parietal endocardium bulged into the left ventricular cavity. The thrombi were reddish brown, showed irregularly contracted surfaces and exhibited on the cut section a layered appearance. One of these animals had concomitant involvement of the tricuspid valve, the anterior leaflet of which was hemorrhagic and edematous in appearance.

The 6 other rabbits, dying from two to five hours after the intravenous injection of the filtrate, displayed similar pericardial and myocardial changes, and in 2 of these mural thrombi were implanted on the wall of the left ventricle.

12. Gerber, I. E.: Arch. Path. **21**:331 and 776, 1936.

Fig. 1.—*A*, parietal endocardial thrombosis, venous dilatation and engorgement, and necrotic-hemorrhagic myocardial change, with polymorphonuclear leukocytes infiltrating the tissues, occurring at the site of an intracardial injection of diluted bacterial infiltrate twenty-four hours after an intravenous injection of the same filtrate—the cardiac Shwartzman phenomenon. (Photomicrograph, ocular 10, objective 8, Zeiss.)

B, mural thrombosis, severe necrotic-hemorrhagic arteritis and periarteritis in a doubly ligated tract of femoral artery occurring at the site of an intraluminal injection of diluted bacterial filtrate twenty-four hours after an intravenous injection of the same filtrate—the intra-arterial Shwartzman phenomenon. (Photomicrograph, ocular 10, objective 8, Zeiss.)

Figure 2
(See legend on opposite page)

Microscopic Changes.—Widespread congestion of capillaries and venules, with recently extravasated red blood cells extensively infiltrating the adjacent tissues, was striking both in the myocardium and in the pericardium. Numerous small and middle-sized veins were thrombosed. The thrombi varied from parietal clumps of hematic cells embedded in the meshes of a fibrinous material to larger amorphous eosinophilic masses obliterating the lumen almost completely. The vessel walls displayed severe necrobiotic changes and abundant leukocytic infiltration, most severely affecting the media and the adventitia. Intimal damage was apparent in the thrombosed tracts of blood vessels, but aside from these areas, in the majority of the vessels encountered the intima appeared fairly well preserved, with endothelial lining and elastic structures being clearly made out. In the hemorrhagic areas, the myofibrils displayed striations and nuclear patterns that were hardly recognizable, and often they appeared fused into amorphous blocks of structureless eosinophilic material. Identical necrobiotic myocardial changes were noticeable at times in other areas apparently free from hemorrhagic infiltration. Proliferating histiocytes, macrophages containing fragments of red blood cells and overwhelming numbers of infiltrating polymorphonuclear leukocytes were constant additional observations. Few leukocytes appeared well preserved; the majority showed karyorrhexis and karylolysis resulting in a scattering of cellular debris. The endocardial parietal thrombi consisted mostly of masses of eosinophilic material which merged indistinctly into the underlying necrotic structureless myocardium (fig. 1*A*). In the anterior leaflet of the tricuspid valve, which had grossly appeared hemorrhagic and edematous, the histologic preparations revealed focal areas of necrosis in the ground substance with loss of fibrillar structure and scattering of cellular debris. Widespread extravasations of red blood cells and diffuse infiltration by polymorphonuclear leukocytes almost completely obliterated the usual valvular structure (fig. 2*A*).

4. Effect of a Local Periarterial and an Intra-Arterial Injection.

All of the control experiments described in the demonstration of the Shwartzman phenomenon in the heart were repeated in the study of the phenomenon in a peripheral artery. After repeated trials, a dilution of the filtrate was obtained which on local injection, either intravascular or perivascular, and on intravenous injection evoked a negligible tissue response in the tract of artery subjected to the double ligature. A dilution of 1 : 30 was found to be satisfactory for local injection and a dilution of 1 : 20 for intravenous administration.

5. Effect of a Local Periarterial Injection Followed by an Intravenous Injection of Bacterial Filtrate.

The pattern of lesions in all 3 animals used was quite comparable to that observed in the cardiac phenomenon and made it unnecessary to study the morphologic aspects of the arterial phenomenon in a larger number of animals.

At the prepared tract of femoral artery the periadventitial fibrous connective tissue appeared edematous, with a mottling of reddish brown discoloration and

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Fig. 2.—*A*, acute hemorrhagic valvulitis (tricuspid leaflet) occurring twenty-four hours after an intravenous injection of diluted bacterial filtrate in an animal subjected to intracardiac preparation. (Photomicrograph, ocular 5, oil immersion, Zeiss.)

B, hyperplasia of the endothelial lining and swelling of the ground substance of a mitral leaflet of an animal killed twenty-four hours after a single intravenous injection of undiluted bacterial filtrate. (Photomicrograph, ocular 10, objective 40, Zeiss.)

pinpoint hemorrhages which involved the vessel wall to a lesser extent. The lumen was patent, and no evidence of thrombosis could be seen.

On microscopic examination the outstanding abnormality was a mural hemorrhagic-necrobiotic change affecting the periadventitial fibrous connective tissue, the adventitia and the external aspect of the medial coat. Concomitantly there was a diffuse infiltration of polymorphonuclear leukocytes, and several vasa vasorum showed fresh thrombotic masses in their lumens. Similar changes were observed in the walls of adjacent veins. Both in the arteries and in the veins the intimal damage was negligible, the evidence consisting mostly of swelling of the collagenous fibers and some increase of subendothelial histiocytes.

6. Effect of a Local Intra-Arterial Injection Followed by an Intravenous Injection of the Filtrate.

Three animals were subjected to the procedure. As in the 3 animals of the previous group, a hemorrhagic change affecting both perivascular fibrous connective tissue and vessel wall was observed in all, together with thrombotic occlusion of the lumen of the prepared tract of artery.

The microscopic sections showed the intraluminal thrombi to consist of masses of amorphous eosinophilic material in which platelets, white blood cells and erythrocytes were enmeshed. Wherever the thrombi were implanted, the underlying intima had lost all structural details. The internal elastic membrane was intact, however. The media and the adventitia displayed marked leukocytic infiltration around foci of tissue necrosis (fig. 1 *B*). The adventitia was widely infiltrated by recently extravasated red blood cells, which also invaded the surrounding periadventitial tissue. Aside from the areas of luminal thrombosis, the endothelial cells lining the intima seemed to be well preserved, and the only evidence of damage consisted of moderate endothelial swelling and interstitial edema. Extension of the inflammatory process from the artery to the vein was observed in 1 instance. The wall of the vein was infiltrated by polymorphonuclear leukocytes, and the lumen was completely occluded by a recent thrombotic mass.

7. The Combined Arterial and Cardiac Shwartzman Phenomenon.

By the double injection technic, in 3 of 4 trials the phenomenon was simultaneously elicited in the intraluminally prepared tract of femoral artery and in the prepared heart. In addition to the striking hemorrhagic-necrobiotic changes in both areas, thrombosis occurred at the arterial site in 2 instances. In a third animal the necrobiotic-hemorrhagic inflammatory reaction was seen to extend from the artery to the adjacent vein, both showing thrombotic occlusion of the lumen. In the fourth animal, the heart displayed the usual pattern of the phenomenon, while the change in the artery was limited to minimal periadventitial lymphocytic infiltration, with occasional granulocytes.

EFFECT OF RHEUMATIC BLOOD ON THE HEART AND FEMORAL ARTERY

Gross Findings.—In none of the animals subjected to this experiment were grossly detectable changes noticed either in the heart or in the prepared stretch of femoral artery.

Microscopic Changes.—Microscopic study of the hearts of the animals given a single or two intravenous injections of the rheumatic blood one week apart (group 2) showed generalized proliferation of histiocytes and lymphocytes, most apparent around the walls of small veins. An identical but more pronounced change was seen in the animals which had received the injection of blood directly

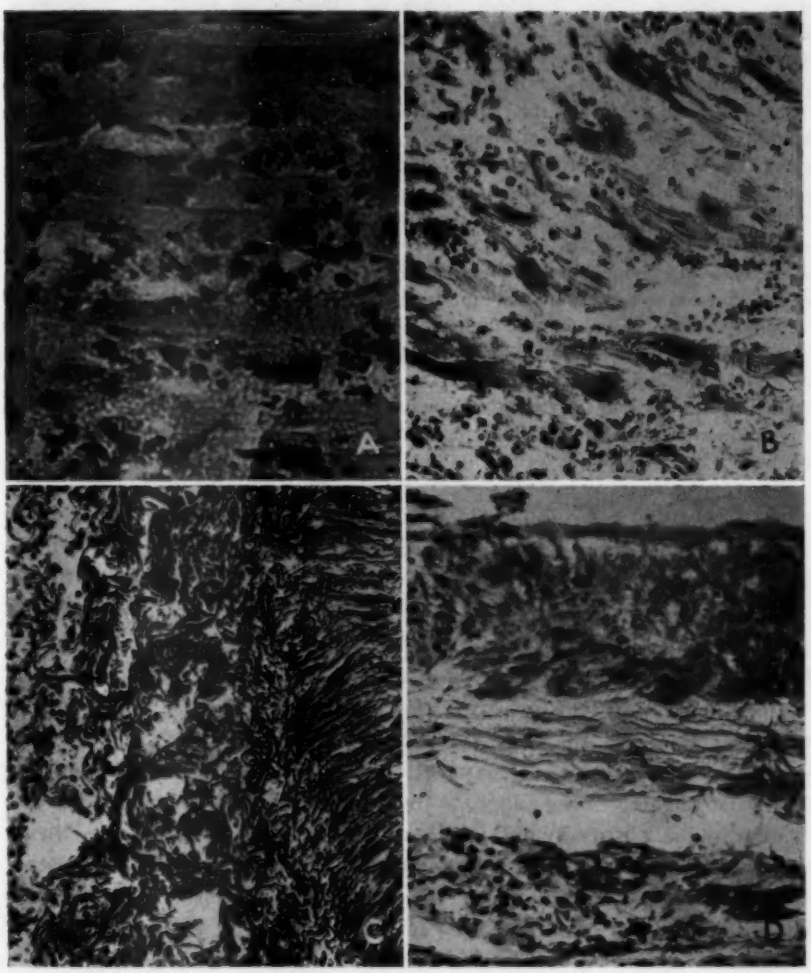

Fig. 3.—*A*, acute myocarditis with diffusely infiltrating polymorphonuclear leukocytes observed twenty-four hours after a single intravenous injection of undiluted bacterial filtrate. (Photomicrograph, ocular 5, objective 40, Zeiss.)

B, myocardial histiocytic hyperplasia in an animal which received two injections of rheumatic blood, one intracardially and the other, twenty-four hours later, in a peripheral vein, and was killed after forty-eight hours. (Photomicrograph, ocular 10, objective, 8, Zeiss.)

C, lymphocytic-histiocytic proliferation occurring in the periadventitial fibrous connective tissue of a doubly ligated tract of femoral artery at the site of an intraluminal injection of rheumatic blood twenty-four hours after an intravenous injection of the same blood. (Photomicrograph, ocular 10, objective 8, Zeiss.)

D, lymphocytic-histiocytic hyperplasia occurring in the periadventitial fibrous connective tissue of a femoral artery of an animal killed twenty-four hours after a single local injection of rheumatic blood. (Photomicrograph, ocular 10, objective 8, Zeiss.)

into the heart (group 1). There was concurrent edema of the periadventitial fibrous connective tissue, in which occasional granulocytes could be recognized. Patchy changes in myofibrils with loss of striations and of cellular outlines were noticed in the myocardium of the animals which received an initial intracardiac injection followed twenty-four hours later by an intravenous injection of rheumatic blood (group 3). Around these degenerated areas there was moderate inflammatory reaction, mostly that of lymphocytes and histiocytes, with scattered granulocytes (fig. 3 *B*). This inflammatory reaction still persisted in the animals killed on the third day, while it seemed to have subsided in the animals examined on the seventh day. In the latter a proliferation of fibroblastic cells was noted.

A fundamentally identical type of tissue response, namely widespread mural and perivascular lymphocytic-histiocytic hyperplasia, accompanied by inconstant and less pronounced granulocytic infiltration, was observed in the ligated tract of femoral artery in the 3 rabbits which had received an intra-arterial injection of a few drops of rheumatic blood and twenty-four hours later an intravenous injection of the same blood (group 5) (fig. 3 *C*). However, both the single local injection (group 4) and the double intravenous injections one week apart (group 2) were productive only of negligible change, characterized by moderate increase of periadventitial mesenchymal cells (fig. 3 *D*).

COMMENT

The changes attending the cardiac and the arterial Shwartzman phenomenon in these animals are similar to those described by others in different organs and tissues under comparable experimental conditions. In contrast with the minimal tissue response on single administration, either local or intravenous, of the properly diluted bacterial filtrate, the striking feature of the process, both in the heart and in the femoral artery, was the hemorrhagic-necrobiotic inflammatory type of tissue reaction at the site of preparation following the intravenous injection of the filtrate. This supports the contention that the factors in the filtrate capable of producing local preparation are distinct from those responsible for inflammation and that the determining factor in the production of the phenomenon is the actual state of tissue preparedness elicited by the local administration of the filtrate.

Control experiments of Gerber [12] have shown that possibly particles of the medium on which the organisms are cultured, the phenolized saline solution in which the filtrate is suspended, and the autolyzed organisms unavoidably present in the filtrate may contribute in some degree to the inflammation attending local preparation. Dialyzed filtrates were used in most of the experiments to lessen the influence of these factors.

Karsner and Moritz,[13] also Kielanowski and Selzer,[14] have found evidence of vascular injury after a single local injection of the filtrate. None of the control animals in the present series which had received a

13. Karsner, H. T., and Moritz, A. R.: J. Exper. Med. **60**:37, 1934.

14. Kielanowski, T., and Selzer, A.: Bull. internat. Acad. polon. d. sc. et d. lett., cl. méd., 1934, p. 417.

single dose of the diluted filtrate either locally or intravenously displayed any evidence of vascular injury, which was the predominant feature at the area of local preparation after intravenous injection of the filtrate. Vascular engorgement, thrombosis and hemorrhage were already present in animals dying two to five hours after the provocative injection. It was not ascertained whether these changes could be seen still earlier.

To explain vascular thrombosis Gerber [12] advocated the concept of Dietrich,[15] namely, "endothelial hyperactivity" initiated by the local preparatory injection and intensified by the provocative factors reaching the vessel walls by way of the blood stream. No histologic evidence of primary endothelial damage was observed in these animals, either in the small vessels within the heart or in the femoral artery, where any endothelial change, if present, could be studied in detail. Both in the prepared femoral artery and in the myocardial blood vessels significant endothelial damage was observed only in connection with extensive mural change and luminal thrombosis.

Loosening of mural structure and vascular thrombosis in a broad area account, most likely, for the hemorrhagic change accompanying the development of the phenomenon at the site of local preparation. In the majority of the animals these hemorrhagic changes were seen to be accompanied by tissue necrobiosis; in some instances, however, necrobiotic change alone was noticed. It affected not only the walls of the blood vessels, the cardiac myofibrils and the intervening stroma but also the granulocytes attending the inflammatory reaction. The granulocytes showed loss of structure within the nuclear confines, karyorrhexis, and karyolysis, with scattering of cellular debris. These leukocytic changes, never observed in the tissue response elicited by the single intravenous injection or by the single local injection of the filtrate, appeared to be so constant in the inflammatory reaction following the double local and intravenous injection of the filtrate as to differentiate the two types of tissue reaction still more and to point to a necrotizing factor within the tissue after preparation, accompanying or perhaps leading to the development of the phenomenon.

* * *

The Shwartzman phenomenon has been considered by some as a variant of the Arthus phenomenon. The immunologic difference between the two reactions has been demonstrated by Shwartzman [1]; the individuality of the two phenomena has been further stressed, on morphologic grounds, by Gerber.[12] According to Gerber, a striking vascular

15. Dietrich, A.: Thrombose; ihre Grundlagen und ihre Bedeutung, in Aschoff, L., and others: Pathologie und Klinik in Einzeldarstellungen, Berlin, Julius Springer, 1932, vol. 4.

response is the chief pattern of the Shwartzman phenomenon, while tissue edema and inflammatory reaction with minimal vascular damage and absence of hemorrhagic-necrobiotic change should be considered the main characteristics of the Arthus phenomenon.

The findings in the series of animals in which the effects of a non-phenomenon-producing substance were studied in the heart and in the femoral artery further point to the morphologic individuality of the Shwartzman phenomenon. The outstanding vascular injury, tissue necrosis and exudative reaction attending the classic phenomenon in the heart and in the femoral artery contrast sharply with the lymphocytic-histiocytic tissue response at the corresponding sites after injection of the non-phenomenon-producing rheumatic blood under identical procedures. The findings in the group of animals receiving rheumatic blood recall those of Seegal [16] in the myocardium of rabbits first submitted to repeated subcutaneous injections of foreign proteins and then given intracardial injections of the same antigens. A similar twofold reaction, histiocytic-leukocytic first and then lymphocytic, is described by Apitz [17] in the heart and the blood vessels of rabbits first sensitized to horse serum by previous repeated parenteral injections and then given injections of the same antigen by the intravenous route. The findings of Klinge [18] in the heart and the blood vessels of rabbits subjected to repeated parenteral injections of horse serum are also similar to those following injection of rheumatic blood. Klinge attributed this type of tissue response to the allergic condition created by the repeated injections of the serum. The fallacy of this conclusion, already demonstrated by a series of experiments (Ravenna [19]; Tedeschi [20]) in which identical changes were produced in a variety of experimental animals by a single parenteral injection of horse serum as well as by single injections of other foreign proteins (milk, frog blood serum), is further shown by the findings in this series of animals which had received single intravenous or intracardiac injections of rheumatic blood.

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Numerous attempts have been made by several investigators to reproduce carditis and angiitis in experimental animals by introducing single or repeated doses of bacterial toxins by various routes. DeVecchi,[21]

16. Seegal, D.; Seegal, B. C., and Jost, E. L.: J. Exper. Med. **55**:155, 1932.

17. Apitz, K.: Virchows Arch. f. path. Anat. **293**:1, 1934; J. Immunol. **29**: 255, 1935.

18. Klinge, F.: Virchows Arch. f. path. Anat. **286**:344, 1932.

19. Ravenna, E.: Sulle reazioni da albumine eterogenee, Cortina d'Ampezzo. Cooperativa Anonima Poligrafica, 1934; Arch. per le sc. med. **37**:236, 1913; **44**:268, 1921.

20. Tedeschi, C. G.: Sperimentale, Arch. di biol. **88**:93, 1934.

21. DeVecchi, B.: Riforma med. **21**:16, 1905.

is accredited with having presented the first experimental pattern of toxic valvulitis, observed following repeated injections of filtrates of staphylococci, streptococci, diphtheria bacilli and typhoid bacilli in animals first subjected to valvular trauma or simultaneously treated with hypertensive adrenal gland extracts. Among the findings he emphasized the patchy necrobiotic changes, edema and swelling of the ground substance of the valve leaflets, accompanied by proliferation of histiocytes and occasional infiltration by polymorphonuclear leukocytes. A pattern of valvular lesions more or less closely fitting the description first given by DeVecchi was produced later by other investigators who repeatedly injected bacterial filtrates over a period of time (Ravenna [19]; Porrini [22]; Mencarelli [23]). Regardless of the type of toxin used in the experiments, degenerative change in the ground substance and proliferation of fixed connective tissue cells were found to be the prevailing patterns.

In the experiments of these investigators myocardial changes were also observed, and in those by others (Scavizzi [24]; Tedeschi [25]; Dietrich [15]), in which the myocardial changes were given main consideration, the predominant features of the tissue response were again patchy degeneration of myofibrils and hyperplasia of connective tissue cells, in the earliest stages mostly lymphocytes and histiocytes and later on fibroblasts, all resulting in more or less pronounced myocardial fibrosis.

If one compares these findings with those observed in the present series in the group of control animals in which single intracardiac injections of progressive dilutions of the bacterial filtrate were tested so as to reach the exact preparatory dose desired to produce the cardiac phenomenon, the more striking inflammatory type of reaction attending the tissue response in this series becomes apparent. The direct delivery of the filtrate into the heart in these experiments, compared with the devious route of introduction of the bacterial toxins (subcutaneous, intravenous) used in the experiments of others, might explain the difference in patterns; however, considering that an identical, although less severe, type of myocardial inflammatory response was seen in this series among the animals which were subjected to single intravenous injections of different dilutions of the filtrate, I think it is a more logical supposition that specific antigenic principles contained in the filtrate prepared according to the "agar washing" method were the main factor in the production of the myocardial exudative response.

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22. Porrini: Virchows Arch. f. path. Anat. **205**:169, 1911.
23. Mencarelli, L.: Cuore e circolaz. **14**:273, 1930.
24. Scavizzi, P. L.: Gior. di batteriol. e immunol. **5**:189, 1930.
25. Tedeschi, C. G.: Gior. di. batteriol. e immunol. **4**:1097, 1929.

Since a systematic investigation of the changes within the endocardium was not the purpose of this study, it was not made in any of the groups of animals: however, thrombosis in the parietal endocardium, with polymorphonuclear leukocytes infiltrating widely, was noticed once in the group of animals which had received a single intracardiac injection of a 1:3 dilution of the filtrate and four times among the animals submitted to the cardiac phenomenon. Gross evidence of hemorrhagic valvulitis was seen in 1 rabbit, but an inflammatory valvular response with definite granulocytes, either sparse or in small groups, was also noticed in leaflets of mitral and tricuspid valves which grossly had appeared to be intact.

* * *

The considerations on the cardiac changes apply also to the changes in the femoral artery. Intraluminal thrombosis with mural hemorrhagic-necrobiotic change, acute angiitis and periangiitis, with polymorphonuclear leukocytes infiltrating widely, were consistently seen, in our rabbits, in the prepared section of femoral artery after intravenous injection of the filtrate. These vascular changes resembled to a certain extent those described by Kusama [26] following repeated injections of vaccines of typhoid bacilli, dysentery bacilli or streptococci. In discussing the findings of Kusama, Siegmund [27] concluded that they might be attributed to endothelial sensitization. It is possible that the active factors in the vaccines used by Kusama were similar in nature to those present in the bacterial filtrates employed in the present experiments; it is questionable, however, whether or not such lesions should be interpreted on the basis of an allergic condition, since both the present observations and those of Apitz [17] and Gerber [12] point to the possibility of similar changes being produced in veins, arteries and capillaries of animals receiving a single intravenous injection of the filtrate.

A vascular pathway between artery and adjacent vein has been shown with injection methods by Winternitz and LeCompte,[28] who succeeded in producing phleboarteritis with extension of the inflammatory process from one vascular system to the other. Examples of apparent close association between disease of artery and disease of its vein are occasionally encountered in autopsy material. The outstanding example, of course, is thromboangiitis obliterans, in which a combination of these changes is the rule. In this connection it is worth mentioning that the inflammatory process extended from artery to vein in several animals of this series.

26. Kusama, S.: Beitr. z. path. Anat. u. z. allg. Path. **55**:459, 1913.
27. Siegmund, H.: Verhandl. d. deutsch. path. Gesellsch. **20**:260, 1925.
28. Winternitz, M. C., and LeCompte, P. M.: Am. J. Path. **16**:1, 1940.

SUMMARY

Local tissue reactivity (Shwartzman phenomenon) was successfully produced in the hearts and femoral arteries of rabbits by the use of "agar washing" filtrates of meningococcus cultures.

Both in the heart and in the femoral artery the changes attending the development of the phenomenon were characterized by a hemorrhagic-necrobiotic, inflammatory type of tissue reaction, which contrasted with the minimal tissue response on single administration, either local or intravenous, of the properly diluted bacterial filtrate. Structural loosening in the vessel walls and vascular thrombosis were constant findings, and most likely accounted for the hemorrhagic change at the site of local preparation. In the majority of the experiments these hemorrhagic changes were seen to be accompanied by tissue necrosis; in some instances, however, necrobiotic change alone was noticed. This type of lesion, never observed in the tissue response elicited by the single local or the single intravenous injection of the properly diluted filtrate, might indicate that a necrotizing factor is present within the tissue after preparation which accompanies or perhaps leads to the development of the phenomenon.

Acute exudative endocarditis, myocarditis and angiitis, characterized by striking granulocytic infiltration, were observed among the control animals in which single intracardiac, intravenous or local arterial (femoral artery) injections of progressive dilutions of the bacterial filtrate were tested, bringing definite evidence of the possibility of eliciting acute exudative inflammatory reactions in the heart and the blood vessels by the use of bacterial toxins prepared according to the "agar washing" methods.

Duplicate experiments in which rheumatic blood was used instead of the bacterial filtrate revealed a tissue response completely different from that elicited in the demonstration of the classic phenomenon, being mainly characterized by a lymphocytic-histiocytic tissue reaction without any vascular change. This further points to the morphologic individuality of the Shwartzman phenomenon.

CHORIONIC GONADOTROPIN IN THE DIAGNOSIS OF TESTICULAR TUMORS

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THE classification of cancers of the testis admittedly is unsatisfactory because many contain a large variety of tissues, both mesoblastic and epithelial, and some have epithelial tissues whose cells may not be identified as regards origin or level of ultimate tissue differentiation. Frequently these tumors are placed in the class of teratoma, one enigmatic term being substituted for another. A wide variety of descriptive terms have been used, and the classifications have varied greatly. Classifications for the most part have been morphologic, but some have been suggested on the basis of pathologic embryology.[1]

New classifications were developed when it was demonstrated that tumors might produce hormone and when gonadotropic hormone was found in the urine of some patients with testicular tumors.[2]

Ferguson [3] advocated a classification based on quantitative determinations of the gonadotropic hormone in the urine. He found that the quantity varied greatly and believed that a specific amount indicated the specific structural type of tumor. Biologic tests were used to make these determinations.[4]

Efforts to identify the type of tumor by qualitative and quantitative determinations of gonadotropic hormone have not clarified the classification, because consideration was not given to the fact that two types of gonadotropic hormone could be present in the urine of these patients and because the significance of each was not properly evaluated. No accurate correlation can be made between hormone determinations and specific type of tissue without consideration of these facts and of

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From the Henry Baird Favill Laboratory and the Department of Gynecology of St. Luke's Hospital and the Department of Gynecology of Northwestern University Medical School.

1. (*a*) Ewing, J.: Surg., Gynec. & Obst. **12**:230, 1911. (*b*) Christopoulas, D. G.: Urol. & Cutan. Rev. **44**:32, 1940. (*c*) Gordon, W. G.: J. Urol. **43**:851, 1940. (*d*) Melicow, W. M.: ibid. **44**:333, 1940.

2. (*a*) Hinman, F., and Powell, T.: J. A. M. A. **110**:188, 1938. (*b*) Twombly, G. H.: Surgery **16**:181, 1944.

3. Ferguson, R. S.: Am. J. Cancer **18**:269, 1933; J. A. M. A. **101**:1933, 1933; J. Urol. **31**:397, 1934.

4. Aschheim, S., and Zondek, B.: Klin. Wchnschr. **6**:1322, 1927.

which tissues are capable of producing the hormones. When these factors are correctly interpreted, the presence of the chorionic type of gonadotropin does indicate a specific tumor, as will be shown later.

Two cases of primary testicular tumor are reported, and a correlation between type of tumor and biologic tests is made. Both patients died from metastases, one thirteen months and the other thirty-seven months after the onset of symptoms.

REPORT OF CASES

CASE 1.—A white man aged 31 years entered St. Luke's Hospital on the service of Dr. Harry Culver, Oct. 13, 1944, in semicoma with complete paralysis of the right hand and arm. He died the following day. The postmortem examination was made by Dr. Edwin F. Hirsch.

The following statements were furnished by the United States Public Health Service: "The patient entered the United States Marine Hospital, Norfolk, Va., May 25, 1944, complaining of a slightly painful, gradual enlargement of the left testicle during the nine months prior to admission. Examination revealed a hard nodular mass the size of a lemon in the left testicle. The reaction to the Friedman test was positive. There was a slight increase in the red cell count and in hemoglobin. May 30 a simple left orchectomy was done. The testicle, 4.1 by 3.0 by 2.1 cm., had attached tunics, spermatic cord and vas; it was hard and gray-white on the external surface, and on cut surfaces an irregular tumor mass had replaced most of the testicular tissue. The tumor had discrete masses of cartilage interspersed with softer, reddish yellow to gray tissues, focally hemorrhagic. It seemed confined within the testicular capsule, and a small amount of soft brown testicular tissue was present at the periphery. The tunics were thickened by a dense fibrous tissue. The cord and the vas deferens were not remarkable; the epididymis was not found.

"*Microscopic Examination.*—Sections from various portions of the tumor contained many varieties of tissue. There were masses of undifferentiated epithelial cells with distinct variation in size, shape and staining quality. The nuclei were large and had prominent nucleoli, and many cells were in mitosis. These cells had no uniformity of structure. Some masses of epithelial cells formed fairly well defined glands of various types, some suggesting renal tubules. There was also an extensive infiltration of highly undifferentiated fibrous tissue, much of it myxomatous and some of it fatty. Many cells in mitosis were in these tissues. Large masses of cartilage were distributed at random through the tumor, and there were many foci of hemorrhage and necrosis and a few with acute inflammatory exudation. The diagnosis was teratoma of testicle (mixed type, of high malignancy). The patient was transferred to the Tumor Clinic, United States Marine Hospital, Baltimore, Md., where between June 14 and July 21 he received 250 kilovolts (peak) of radiation therapy with 2,000 roentgens (r) to each of the following regions: the left side of the epigastrium, the left side of the mid-abdomen, the left groin, the posterior epigastrium and the lumbar region. Roentgenologic examination at that time revealed a number of metastatic pulmonary lesions."

Only the pertinent portions of the necropsy report are given here.

The lower margin of the right lobe of the liver extended 5 cm. below the costal arch in the right axillary line. Along the lower edge of the left lobe of

the liver was an almost continuous mass of hemorrhagic brown and gray tissue that extended for 11.5 cm. along the edge and reached into the liver 2 to 3.5 cm. It had a maximum thickness of 3 cm. There were several similar nodules on the posterior surface of the liver.

There were no adhesions between the right lung and the chest wall, but the lung had many hard nodules, some gray, others hemorrhagic and black, ranging from 1 to 3 cm. in diameter. The same conditions were observed on the left side. The unopened right lung weighed 450 Gm., and the left weighed 390 Gm. Broad surfaces made by cutting both the right and the left lung had nodules of firm and spongy tissues with recent hemorrhages.

The dura mater was a thin, gray-white membrane that stripped from the calvarium without resistance. There was little cerebrospinal fluid, and it was stained with blood. On the left side near the vertex were subpial hemorrhages along the course of the blood vessels. There was a distinct flattening of the convolutions of the brain. In the left cerebral hemisphere near the vertex, 4 cm. from the midline, was a hemorrhagic nodule in the cortex, 1.5 cm. in diameter, and another on the right side, 4 cm. from the posterior portion and 3 cm. from the midline, was 1 cm. in diameter.

The spleen weighed 310 Gm. On the lateral border 4.5 cm. from the lower pole was a nodule 1.5 cm. in diameter which projected 5 mm. above the surface. It extended 1 cm. into the splenic pulp and had an outer hemorrhagic serrated border and a yellow avascular center.

The right testis was 4 by 2.5 by 2.5 cm. On broad cut surfaces the tissue was light tan-brown and stringy. There were no changes in the epididymis. The left testicle was absent.

In the fat tissues below the pericardial sac there were nodules of hemorrhagic and black tissue.

The left kidney was 11.3 by 6 by 4.5 cm. and weighed 190 Gm. without its peripheral fat and capsule. The capsule stripped from a smooth red-brown surface except opposite a nodule of hemorrhagic tumor tissue 2.5 cm. in diameter toward the lower pole. Another hemorrhagic nodule on the anterior surface near the hilus was 2.8 cm. in diameter.

Microscopic Examination.—The various metastatic tumor nodules had similar tissues. In the nodules were large recent and old hemorrhages with red cells, much fibrin and scattered leukocytes. The tumor cells which had diffusely invaded the tissues were in broad and smaller masses and were of two kinds (fig. 1).

Most of the cells were like Langhans cells. They were polygonal and had large vesicular nuclei and coarsely vacuolated cytoplasm. These cells in some places varied in size and appearance approximately as did those observed in implantation sites of the human placenta. In other places the cells had huge deeply stained nuclei, and many were in mitosis. Scattered through these cell aggregates were smaller masses of syncytium in a meshwork about blood spaces. Some had engulfed erythrocytes. The syncytium in some places was spun out; in others it was solid multinucleated cell masses, and in still others it consisted of deeply stained, irregularly shaped cells with single or double nuclei.

The tunica albuginea of the right testis was dense and fibrous. The tubules had relatively thick fibrous capsules and atrophic germinal epithelium. There was little evidence of spermatogenesis. The lumens were empty, and the germinal cells had retrogressive changes. There were a few masses of interstitial cells in the loose vascular connective tissue.

Diagnosis.—Metastatic hemorrhagic choriocarcinoma of the lungs, the brain, the liver, the kidney, the spleen and the mediastinal lymph nodes and atrophy of the right testis.

CASE 2.—A white man aged 22 years entered St. Luke's Hospital Dec. 8, 1942, in the care of Dr. A. R. Morrow. The left testicle had never been in the scrotum; it could be felt as a small mass in the groin and for two months prior to

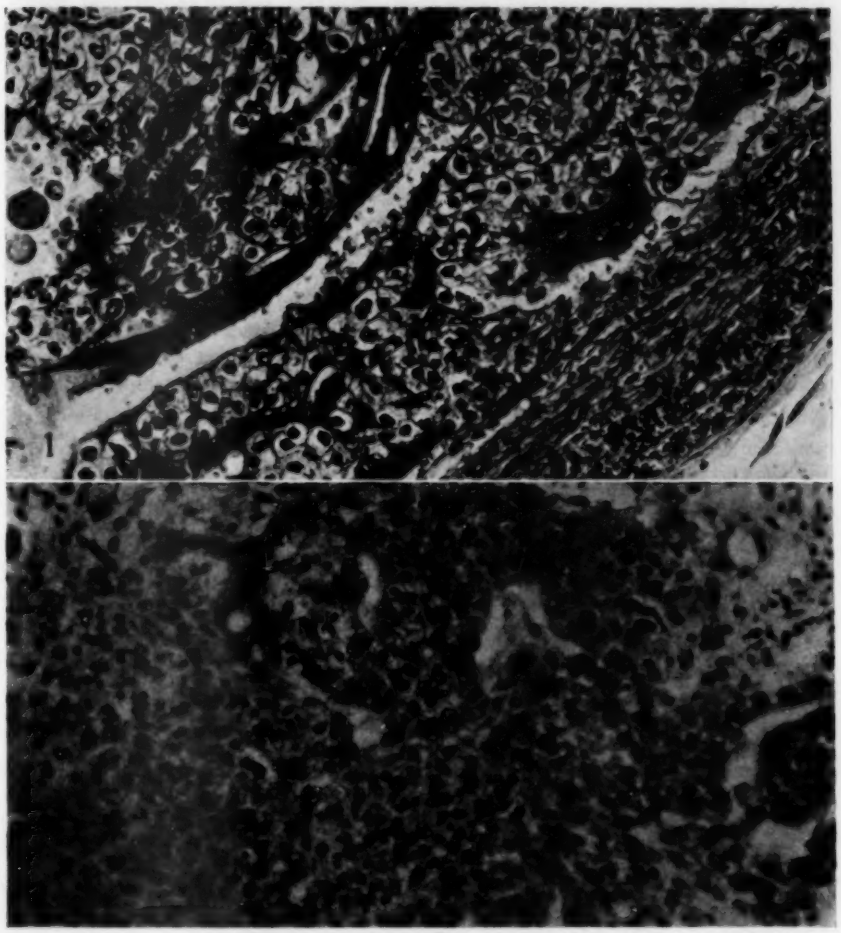

Fig. 1 (case 1).—Photomicrograph of a metastatic lesion in the lung. The cells resemble Langhans cells and syncytium of chorion. × 198.

Fig. 2 (case 2).—Photomicrograph of a primary tumor of the testis. The tumor tissue is arranged in mosaics with some slits or lumen-like structures. There are a few deeply stained multinucleated cell masses. × 198.

admission had increased in size. When he was examined there was a mass the size of a large egg in the left inguinal canal. The left testicle was not in the scrotum.

A simple left orchectomy was done December 10. The body of the left testicle was 4.5 by 3 by 2.5 cm. About two thirds of the substance in the body of the

testis was replaced by gray-yellow tumor tissue extending from the epididymis toward the tunics of the testis. This tumor tissue had a finely nodular appearance and was elevated above the surrounding tissue. There were small yellow regions of necrosis.

Histologic examination of the testicle revealed extensive regions of necrosis and hemorrhages. The tumor tissue consisted of large and small masses of cells arranged in mosaics and also having some tissue crevices or lumen-like structures. The cells were of medium size, with pale cytoplasm and large vesicular nuclei containing coarse chromatin granules. Many cells were in mitosis. There were a few large multinucleated giant cells (fig. 2). In some portions of the testicle the lymph channels and the blood vessels were dilated, and the lumens contained masses of tumor cells. The tissues of the body of the testicle had undergone retrogressive changes. There were many atrophic tubules with and without lining cells. In the stromal tissues of the epididymis there were several masses of tumor cells. The interstitial cells were not abundant.

The diagnosis was seminoma of the left testis with metastases in the funiculus spermaticus.

An Aschheim-Zondek test made nine days after the operation was positive. Roentgenologic examination of the chest on the seventh postoperative day (December 17) revealed no evidence of metastatic lesions in either lung field or in the ribs and no enlargement of the mediastinal glands. The patient made an uneventful recovery and was discharged from the hospital on the tenth postoperative day.

He was readmitted June 12, 1945, for cystoscopic examination and treatment of nonspecific vesiculitis, prostatitis and cystitis. An intravenous pyelogram revealed no abnormality of the kidneys or the ureters.

October 8 the patient was admitted for the third time, with an inguinal hernia on the right side. He complained of pain and swelling of the mammary glands for a period of three months, loss of 30 pounds (13.6 Kg.) in the previous three months, occasional substernal pain and hemoptysis of bright red blood on one occasion in the month prior to this admission.

There was a right indirect inguinal hernia. The breasts were enlarged, tense and tender. The lungs were normal. Roentgen examination of the lungs revealed no abnormality, and there was no change from the previous findings on Dec. 17, 1942. Roentgen examination of the lumbar spine and the pelvis revealed no abnormalities. Herniorrhaphy was done on the right side Oct. 10, 1945, and the patient was discharged October 21.

November 9 the patient was admitted for the fourth time, complaining of progressive weakness, substernal pain that was increasing in frequency and severity, loss of 50 pounds (22.7 Kg.) in six months, cough and daily hemoptysis of bright red blood. Examination revealed retraction of the supraclavicular and infraclavicular spaces, with areas of dulness, and rales and inspiratory wheezes in both lungs. The mammary glands were enlarged.

Roentgenologic examination demonstrated both lung fields studded with multiple small nodular masses so numerous that they appeared to coalesce. An intravenous pyelogram showed the left kidney and the upper part of the ureter displaced away from the midline. The blood had 7.5 Gm. of hemoglobin per hundred cubic centimeters and 2,900,000 red blood cells and 8,650 leukocytes per cubic millimeter. Results of all other clinical and laboratory examinations were normal or negative. An Aschheim-Zondek test was not made at this time.

The clinical diagnosis was metastases of the lung from the primary seminoma of the left testicle surgically removed in 1942.

The patient died November 20. The postmortem examination was made the same day by Dr. Edwin F. Hirsch. The essentials are as follows:

Both lungs were noticeably distended and had multiple firm nodules. On cut surfaces these nodules were porous, hemorrhagic and gray-red, ranging in size to 2.5 cm. The right lung weighed 1,835 Gm. and the left 1,635 Gm.

The lymph nodes at the bifurcation of the trachea and the main bronchi were together in a mass 5.5 by 3 by 1.5 cm. On cut surfaces they had edematous red-brown lymph node tissue. In front of the esophagus just below the bifurcation were gray-red lymph nodes on both sides, the largest 5 by 2 by 1.2 cm. They were composed of firm, red-brown, blackened tissues. Around the abdominal aorta were many large lymph nodes also with spongy hemorrhagic tissue. The periaortic, mediastinal and mesenteric lymph nodes were similar.

Beneath the capsules of both kidneys were many small hemorrhagic nodules from pinpoint to 8 mm. in diameter. On surfaces made by hemisecting the kidneys there were small hemorrhagic lesions as large as 8 mm. scattered in the cortex.

The body of the right testis was 5.5 by 3.5 by 2.5 cm. and was composed of tan-brown stringy tissue.

Both mammary glands were enlarged. The right was 7.5 cm. in diameter and 1.5 cm. thick, and the left was 6.0 cm. in diameter and 1.5 cm. thick. At one place in the right gland was a cyst or dilated duct 4 mm. in diameter. Also in the breast tissue was a hemorrhagic tumor nodule 6 to 8 mm. in diameter.

The adrenal glands were of the usual size. The cortex of each was yellow with an abundance of lipid.

Microscopic Examination.—All of the metastatic lesions were similar in tissue structure. Most of the cells were in masses and resembled those of the cytotrophoblast (fig. 3). They were polygonal and had vesicular nuclei and pale, coarsely vacuolated cytoplasm. The nuclei in some were huge and darkly stained. There were also masses of syncytium with eosinophilic, vacuolated cytoplasm containing many large nuclei. In some regions the syncytium was characteristically spun out (fig. 4). There were extensive regions of hemorrhage extending into the adjacent tissues and large regions of necrosis with exudates of polymorphonuclear leukocytes, lymphocytes and large mononuclear phagocytes.

The mammary gland tissues consisted mainly of edematous fibrous stroma and dilated duct structures lined by columnar epithelium. The duct epithelium was moderately hypertrophied.

Diagnosis.—Metastatic choriocarcinoma of the lungs, the kidneys, the right mammary gland and the tracheobronchial, periaortic, mesenteric and mediastinal lymph glands and hypertrophy of the mammary glands.

COMMENT

These two tumors in their primary sites in the testicles were histologically different. In the first instance histologic identification of the various tissues led to a diagnosis of teratoma, while in the second, classification of the tissues justified only the diagnosis of seminoma. In both, however, the metastases were choriocarcinoma. When each patient was first seen with the primary testicular tumor, biologic tests for chorionic gonadotropin were positive. This suggests that the primary tumors had some similar features.

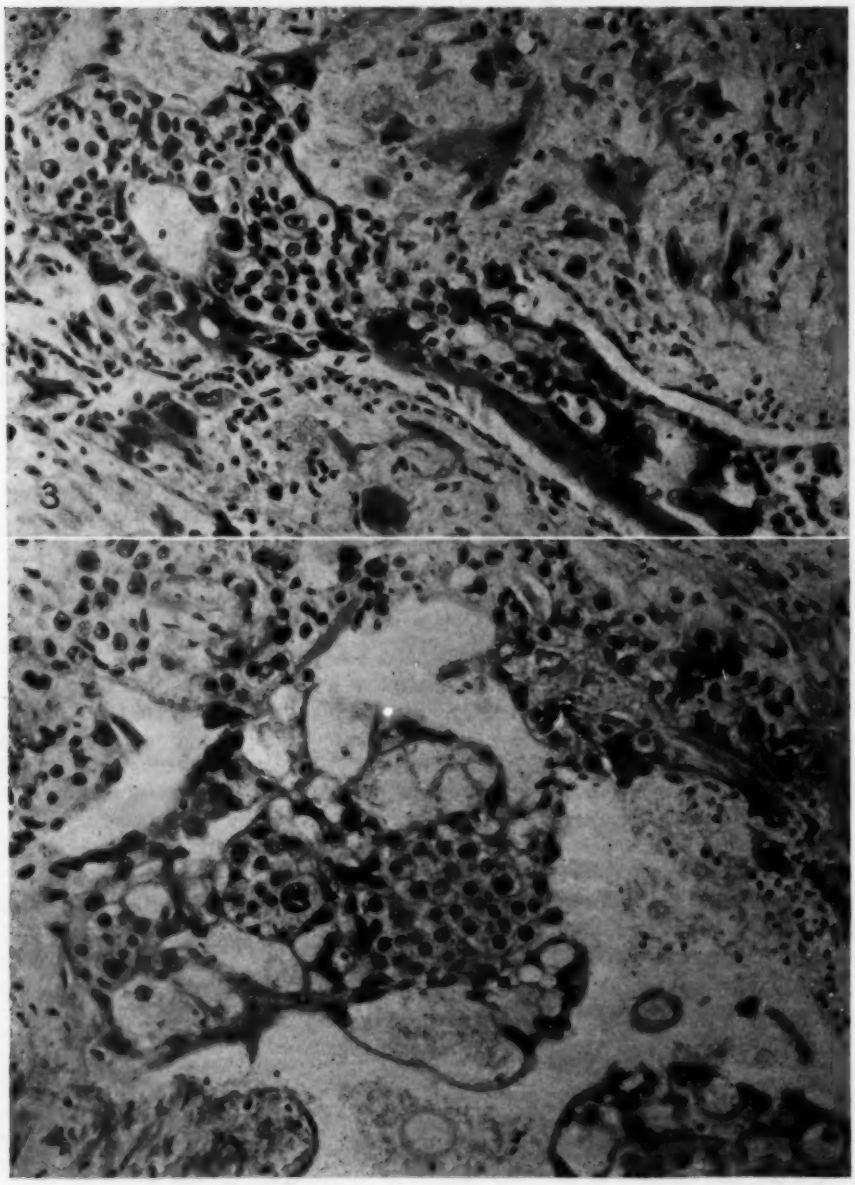

Fig. 3 (case 2).—Photomicrograph of a metastatic lesion in retroperitoneal tissues. The cells resemble Langhans cells of the chorion. There are masses of syncytium. × 198.

Fig. 4 (case 2).—Photomicrograph of a metastatic lesion in retroperitoneal tissues showing "spun-out" syncytium. × 198.

The biologic test described by Aschheim and Zondek [4] has been made in cases of various types of testicular tumors by numerous workers. It was positive in some but not all cases and it has been reported both positive and negative with the same histologic type of testicular tumor, so that its significance has been variously interpreted.[5] Some reports stated that the test is positive in cases of teratoma and seminoma, while others declared that it is negative in such cases. It is generally accepted that the quantitative test advocated by Ferguson [3] is not a reliable means of differentiating types of testicular tumors. Two distinct types of gonadotropin test reactions have been observed in cases of testicular tumor. These two reactions represent two different gonadotropic hormones. These two types of hormones and their biologic and chemical differentiation have been described by many workers.[6] One hormone is the chorionic type of gonadotropin that is identical with that found in the urine of pregnant women. It produces luteinization in the ovarian follicles of the test animals. The other is the castrate type that is elaborated in the pituitary gland and is found in castrates, male and female, in women past the menopause and in elderly men. This hormone produces follicle growth but not luteinization in the ovaries of the test animals.

In cases of testicular tumor distinction should be made between these two reactions. Such differentiation was not possible in earlier reports because the reactions were not at that time completely understood and accepted. Confusion has occurred in the correlation of the test and the type of tumor because of failure to differentiate properly these two test reactions. Chronic gonadotropin in the urine indicates the presence of a biologically active tissue. The presence of the castrate type of gonadotropin, on the other hand, indicates only that there is reduction or cessation of testicular function. The demonstration of this type of hormone does not in any way indicate the presence or the absence of a biologically active tumor tissue.

Further confusion has occurred as a result of a lack of a uniform histologic interpretation of a given tumor tissue and a lack of a uni-

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6. Evans, H. M., and Simpson, M. E.: Am. J. Physiol. **89**:371, 375, 379 and 381, 1929. Engle, E. T.: J. A. M. A. **93**:276, 1929. Hamburger, C.: Acta path. et microbiol. Scandinav., 1933, supp. 17, p. 1; Compt. rend. Soc. de biol. **112**:99, 1933. Selye, H.; Collip, J. B., and Thompson, D. L.: Endocrinology **17**:494, 1933. Engle, E. T.: Am. J. Physiol. **106**:145, 1933. Leonard, S. L.: Proc. Soc. Exper. Biol. & Med. **30**:403 and 1251, 1933; Anat. Rec. **55**:26, 1933. Witschi, E., and Keck, W. N.: Proc Soc. Exper. Biol. & Med. **32**:598, 1935. Liu. S. H., and Noble, R. L.: J. Endocrinol. **1**:7 and 15, 1939.

versally accepted nomenclature and classification of tumors. For example, some authors described embryonal carcinoma and seminoma as one and the same tumor, while others classified them as separate and distinct pathologic entities. Attempts by these authors to correlate the results of biologic tests with types of tumor could lead only to confusion.

It is apparent that in attempting a correlation the various workers have first made a diagnosis of tumor type based on certain established histologic criteria. The biologic test is then stated to be either positive or negative in association with the histologically determined type of tumor. Thus, if the tumor is diagnosed histologically as teratoma, the test is said to be positive or negative for teratoma. The correlation is not carried farther. The identity of the specific tissue in the tumor which might produce the hormone has not been adequately considered.

As a result of the facts noted, the literature contains diverse and conflicting data, the true value and the significance of the Aschheim-Zondek test has been lost, and progress toward identification, classification and understanding of testicular tumors has been retarded.

The chorionic gonadotropin is the more important of the two hormones mentioned in instances of testicular tumor. Several pertinent facts concerning the production of this hormone deserve consideration.

This hormone is produced by the cells of the fetal placenta.[7] Jones, Gey and Gey,[8] working with in vitro cultures of cells from the human placenta and choriocarcinoma, made assays and demonstrated the presence of this hormone after the mediums had been changed several times and when the explants consisted of only new cells. They showed thus conclusively that these cells produced the chorionic gonadotropin.

In early pregnancy, at the time when the chorionic gonadotropin appears in the urine, the growth of the chorionic tissue greatly exceeds the development of the embryo proper. The tissues of the embryo itself are in such an early stage of organization that it is not conceivable that they have the ability to produce a hormone.

As the embryo develops toward its final stage and its glands and organs are more differentiated, the urinary chorionic gonadotropin titer is diminished. Most authors agree that the urinary level of this hormone reaches a peak by the sixtieth to the eighty-fourth day after the first day of the last menstrual period and then falls sharply.[9] At about

7. Bischoff, F.: Endocrinology **30**:525, 1942. Collip, J. B.: Canad. M. A. J. **22**:761, 1930. Ware, H. H.; Main, R. J., and Taliaferro, I.: J. A. M. A. **111**:524, 1938.

8. Jones, G. E. S.; Gey, G. O., and Gey, M. K.: Bull. Johns Hopkins Hosp. **72**:26, 1943.

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this time the Langhans cells, which are a part of the chorionic tissue, begin to disappear. During the remainder of the pregnancy the level is low. These facts suggest that the tissues of the fetus proper do not produce the hormone.

In some instances of abortion and tubal pregnancy in which the chorionic tissues survive and continue to grow for some time, the chorionic gonadotropin continues present in the urine although the embryo itself has been absorbed. This indicates that tissues of the embryo proper do not produce the hormone.

A well established fact is that in the human female choriocarcinoma is associated with high titers of urinary chorionic gonadotropin in the absence of any tissues of the embryo proper. It is also known that after the removal of a choriocarcinoma of the uterus the chorionic gonadotropin disappears in the absence of metastases. When metastases occur the chorionic gonadotropin reappears in the urine. In these instances no embryo is present. Accordingly the tissues of the embryo proper do not produce this hormone.

The chorionic gonadotropin in the urine of patients with testicular tumors or metastases of these is identical with the chorionic gonadotropin found in the urine of pregnant women,[10] and the view generally accepted is that the hormone is produced by the tumor tissues.

All the evidence, both clinical and experimental, indicates that the chorionic gonadotropin is produced by chorionic tissues, that the tissues of the embryo proper do not produce the hormone, that no other tissues are known to produce it, that when the hormone is present in instances of testicular tumor it is identical with the chorionic gonadotropin found in the urine in pregnancy and that in these instances it is produced by the tumor tissues.

It has been accepted that a tumor tissue can have a hormone-producing function, as exemplified by the granulosa cell tumor and arrhenoblastoma.[11] Each of these tumors may vary greatly in histologic structure. Especially in arrhenoblastoma there frequently are multiple types of tissue. The hormone produced by this tumor is not considered to originate in all the varied tissues but rather is believed to be produced by a specific type of tissue that is but a part of the tumor. In this respect arrhenoblastoma is comparable to a tumor of the testicle in which multiple tissues are present. If such a tumor should produce a hormone, and not all of them do, clinical experience and experimental evidence indicate that the hormone would arise from a specific tissue and not from all the types of tissue found in the tumor.

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10. Fevold, H. L.; Fiske, V. M., and Nathanson, I. T.: Endocrinology **24**: 578, 1939.

11. Meyer, R.: Am. J. Obst. & Gynec. **22**:697, 1931.

Rather than to go far afield and believe that testicular teratoma as such, or embryonal carcinoma, or seminoma produces the chorionic gonadotropin, it would seem more consistent to believe that when the chorionic gonadotropin is present, sufficient chorionic tissue is present in the tumor to produce this hormone. A small amount of such tissue may elaborate sufficient quantities of the hormone to cause a biologic test to be positive. This is true for choriocarcinoma of the female, and it has also been observed in the case of a small testicular tumor.[12]

That these tissues may not be recognized in microscopic examinations is understandable. The difficulty of positively identifying chorionic cells microscopically is well known. In fact, many believe that a microscopic diagnosis of choriocarcinoma is not justified without demonstration of the chorionic gonadotropin. It is possible that chorionic tissues are present in some testicular tumors but cannot as yet be identified histologically. Another possible source of failure to diagnose chorionic tissue in a tumor is that only relatively few blocks of tissue are taken from portions of the tumor for microscopic study. Chorionic tissue might not be present in the blocks chosen for examination. Since these tissues need be only small in amount, they could be overlooked unless many more blocks were taken and more sections were made.

In the 2 instances reported here the fact that chorionic gonadotropin was demonstrated in the urine at the time the primary tumor was found indicated that the tumor contained tissue capable of producing the hormone. Since only chorionic tissue produces this hormone, chorionic tissue must have been present in the primary tumor. Microscopically, however, no chorionic tissue was identified in the sections of the tumor in the first instance, and in the second the presence of a few large cells was not considered sufficient for the diagnosis of choriocarcinoma (fig. 2). On the basis of histologic criteria a diagnosis was made in the one instance of teratoma and in the other of seminoma. Since the correlation of the results of the biologic test and types of testicular tumor was in such a confused state, the essential significance of the positive results of the test in these 2 instances was not completely evaluated. In view of the facts which have been presented, the presence of chorionic gonadotropin in the urine should have indicated that chorionic tissue was present in the primary tumor, in the one instance in association with a teratoma and in the other with a seminoma.

That this contention is true is proved by the fact that the metastases in both instances were choriocarcinoma (figs. 1, 3 and 4). For such a type of metastasis to occur, choriocarcinoma must have been present in the primary tumor. There is no reason to believe that choriocarcinoma was primary in the multiple sites in which it was found in both

12. Craver, L. F., and Stewart, F. W.: J. A. M. A. **106**:1802, 1936.

patients. There is no evidence that it had an extragenital origin. Rather one is certain that in each instance it had metastasized from the surgically removed testicular tumor even though chorionic tissue was not observed in this tumor. The presence of the chorionic gonadotropin in both instances indicated the true nature of the primary tumor and the chorio-carcinomatous metastases subsequently proved this to be correct.

Thus, based on the positive biologic test for chorionic gonadotropin, the accurate and complete diagnosis of the primary testicular tumor would have been teratoma with choriocarcinoma in the first instance and seminoma with choriocarcinoma in the second.

It is entirely conceivable that so-called teratoma, seminoma and other types of testicular tumors may occur with or without choriocarcinoma. If choriocarcinoma is not present, the test for chorionic gonadotropin would be negative and if present the test would be positive. The confusion that exists because the test is positive in some cases of teratoma, seminoma, etc., and negative in others would thus be explained.

SUMMARY

Differentiation of the chorionic gonadotropin and the castrate type of gonadotropin, both of which may be present in the urine of patients with testicular tumors or metastases of these, or both, is essential.

Chorionic gonadotropin in the urine of a patient with a testicular tumor indicates that there is present in the tumor a hormone-producing tissue. This tissue is chorionic tissue.

The chorionic tissue may not be demonstrated in the primary tumor because it may be but a small part of the growth, because the entire tumor is not examined microscopically or because it is impossible microscopically to differentiate chorionic tissue from the multiple and varied tissues of a testicular tumor.

In the 2 cases presented here a test of the urine for chorionic gonadotropin indicated that chorionic tissue was present in the primary tumor even though such tissue was not identified microscopically. That chorionic tissue was present was subsequently proved by the metastases, which in each instance were choriocarcinoma.

If chorionic gonadotropin is present in the urine of a patient with a testicular tumor, it indicates the diagnosis of choriocarcinoma even though chorionic tissue is not identified microscopically in the primary tumor.

TOXICITY AND DETOXICATION OF CINCHOPHEN

Experimental Studies

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DESPITE a great deal of clinical, pathologic and experimental work in the past,[1] several important pharmacologic and toxicologic aspects of the action of cinchophen on the organism of man have remained controversial or obscure. This lack of established knowledge is particularly disconcerting because of the alleged injurious effect of cinchophen on the function and on the parenchyma of the liver and because of the resulting serious interference with the institution of effective countermeasures.

The following experiments were undertaken in an attempt to obtain additional data on the hepatotoxic action of cinchophen and on conditions possibly favoring or counteracting such an effect.

EXPERIMENTS

Influence of Cinchophen on the Prothrombin Time of Rats.—Rawls[2] found that the incidence of the various toxic symptoms elicited by cinchophen in man could be considerably reduced by simultaneous administration of vitamin K, which, it was claimed, improved the function of the liver. On the basis of such considerations Rawls proposed the use of the prothrombin time test as a criterion of the function of the liver in persons receiving cinchophen. It seemed to be pertinent, therefore, to determine whether or not cinchophen, like salicylates, when given in excessive doses to rats kept on a diet low in vitamin K causes lengthening of the prothrombin time.

Thirty rats weighing about 125 Gm. were placed on a vitamin K–deficient diet, composed of 18 parts of casein, 8 parts of yeast, 4 parts of salt, 5 parts of cottonseed oil, 2 parts of cod liver oil and 63 parts of cerelose. The rats were allowed to eat this stock diet ad libitum during the day. During the night they were offered 5 Gm. of a corn starch-cotton seed oil mixture containing 100 mg. of cinchophen, which they usually consumed. This management was continued for six weeks. The prothrombin time was determined with Quick's method, a 12.5 per cent plasma dilution being used, before the special dietary treatment was started and at weekly intervals thereafter.

The prothrombin time, which fluctuated between 23 and 30 seconds, average 27.5 seconds, at the start of the experiment, ranged from 39 to 98 seconds, average 60.5 seconds, at the end. The rats were then placed on the stock diet, which

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From the Warner Institute for Therapeutic Research.,

1. Footnote deleted by the author.
2. Rawls, W. B.: New York State J. Med. **42**:2021, 1942.

was fortified with 20 mg. of menadione (2-methyl-naphthoquinone) per day. After three weeks of this diet the prothrombin time had dropped to 15 to 20 seconds, average 17.5 seconds.

Cinchophen, which has a certain pharmacologic resemblance to salicylates, thus causes lengthening of the prothrombin time in rats kept on a diet low in vitamin K.

Effect of a Low Protein, Low Methionine Diet on the Toxicity of Cinchophen.—It is known from clinical and experimental studies that a systemic deficiency of protein, caused by insufficient intake of protein or excessive loss of protein, reduces the resistance of the liver to typical hepatotoxins, such as phosphorus and chloroform. Glynn and Himsworth [3] reported that such a diet, deficient in methionine, when given to rats may elicit extensive, hepatic degenerations which closely resemble the changes of acute yellow atrophy of the human liver. Inasmuch as it has been maintained that malnutrition represents an important factor favoring the development of cinchophen hepatitis in man, it was decided to feed cinchophen to rats kept on a diet with a low protein (2 per cent), low methionine content. Gelatin was used as the exclusive source of protein, as this substance contains only 0.8 per cent of methionine. The following diet was used:

Sucrose	25,300	Gm.
Gelatin	1,500	
Salts	1,200	
Corn oil	600	
Vitamin B_1	150	Mg.
B_2	300	
B_6	150	
Nicotinic acid	3	Gm.
Choline	6	
Calcium pantothenate	3	
Inositol	6	
Para-aminobenzoic acid	3	
Ascorbic acid	3	
Cod liver oil	600	
Total	30,600	Gm.

Thirty rats weighing about 150 Gm. received this diet only, while a second set of 30 rats was fed this diet supplemented with cinchophen (180 Gm. of cinchophen in 15 Kg. of the diet). Each rat was given 10 Gm. of this food daily, containing 0.06 Gm. of cinchophen.

The rats of both groups rapidly lost weight. At the end of a ten week period the average weight of the control group had dropped to 82.8 Gm. and that of the group with cinchophen added to the diet, to 69 Gm. Moreover, there were 17 deaths in the cinchophen group at the end of the experimental period against 4 deaths in the control group. The toxic effects of cinchophen became further manifest in an earlier and more marked appearance of brown incrustations of the hairs of the nape, the ears and the snout in the cinchophen series. These incrustations were present in 27 rats of the cinchophen group, in which they were in some instances of a very advanced type, while they were observed in only 13 of the control group, in which in general they were of a mild to moderate type.

These manifestations, suggesting the presence of a vitaminic deficiency, were not overcome by doubling or later tripling the daily intake of the vitamins composing the vitamin complex contained in the diet. The same failure resulted when 5 rats showing these incrustations were fed the experimental diet reenforced

3. Glynn, L. E., and Himsworth, H. P.: J. Path. & Bact. **56**:297, 1944.

with calcium pantothenate, riboflavin or thiamine, respectively (100 Gm. of the diet plus 100 mg. of calcium pantothenate, 2.5 mg. of riboflavin or 26 mg. of thiamine hydrochloride).

The postmortem examinations of the rats which died during the course of the experiment and which were killed at the end of it, in both series, did not reveal any gross evidence of hepatic degeneration or necrosis. The histologic study of the livers revealed the fact that in the rats of the control series the cytoplasm of the liver cells was highly rarefied and there was mild to moderate interstitial edema, while in the cinchophen series the liver cells were of almost normal appearance. Rarefaction of the cytoplasm was rarely seen. There was some pyknosis, as well as mild to moderate interstitial edema.

The observations thus made confirm those previously reported by other investigators (Barbour and Gilman [4]) showing that restriction of the caloric intake and of the consumption of protein does not favor the production of diffuse and acute necrosis of the liver in animals fed cinchophen. They also demonstrate that a distinct hepatoxic effect of the type of acute yellow atrophy is not obtained when a diet low in protein and in methionine is given in association with cinchophen.

Effect of Cinchophen on Monkeys.—In view of the repeated failure to produce in mice, rabbits, rats, guinea pigs, cats and dogs, by oral or parenteral administration of toxic doses of cinchophen, hepatic lesions resembling those of acute yellow atrophy of the human liver, it appeared desirable to test monkeys in this respect. Two adult Macacus rhesus monkeys, weighing 8 Kg. and 6.3 Kg., respectively, were given daily, by stomach tube, a suspension of 2 Gm. and 1.5 Gm. of cinchophen, respectively, in a 0.5 per cent tragacanth solution. One of the monkeys died after sixteen days of this management, diarrhea, weakness and somnolence having developed. The second monkey died after having lived forty-six days. He appeared to be normal during the early part of the experiment but subsequently became increasingly pale, nervous, cold and weak, and passed loose, soft stools. His appetite varied from fairly good to poor during the major portion of the experimental period. During the last days he refused food, vomited after the introduction of the cinchophen-tragacanth suspension and could scarcely be roused from his severe somnolence.

The autopsies of both monkeys revealed normal stomachs. The livers were congested and exhibited a normal structural pattern. The lungs were hyperemic and mildly edematous.

The results of this experiment show that monkeys do not seem to differ in their hepatic reactivity to cinchophen from other species, and provide thereby additional evidence for the concept that cinchophen is not a hepatotoxic chemical in the ordinary sense.

Acute Experiments in Detoxication.—Among the various detoxicating agents (sulfuric acid, acetic acid, choline, glycuronic acid, glycine, glutamic acid, cysteine, methionine and others) two (glycuronic acid and glycine) have been mentioned in connection with cinchophen detoxication. Lutwak-Mann [5] stated that rat liver does not show any ability to form conjugates of glycuronic acid with cinchophen. Quick [6] suggested that the detoxication of cinchophen in the body probably results from its being combined with glycine.

4. Barbour, H. G., and Gilman, A.: J. Pharmacol. & Exper. Therap. **55**:400, 1935.

5. Lutwak-Mann, C.: Biochem. J. **36**:706, 1942.

6. Quick, A. J.: J. A. M. A. **99**:1190, 1932.

In the experiments to be reported cysteine hydrochloride, ascorbic acid and choline dihydrogen citrate were chosen as detoxicants and were given to mice which received cinchophen by mouth. From preceding experiments it was known that 25 mg. of cinchophen suspended in a mucilaginous vehicle and administered by stomach tube to a mouse weighing 25 Gm. exerts, as a rule, a lethal effect in 50 per cent of the mice. In the first experiment the following 4 groups of mice were used: 30 mice, each receiving 25 mg. of cinchophen; 30 mice, each receiving 25 mg. of cinchophen plus 25 mg. of ascorbic acid; 30 mice, each receiving 25 mg.

TABLE 1.—*Effect of Certain Natural Detoxicants of Cinchophen on the Mortality Rate of Cinchophen-Treated Mice*

Medication	Mice Dead	
	4.5 Hr.	24 Hr.
Cinchophen	15	15
Cinchophen + ascorbic acid	5	6
Cinchophen + cysteine hydrochloride	19	21
Cinchophen + ascorbic acid + cysteine hydrochloride	18	20

of cinchophen plus 25 mg. of cysteine hydrochloride; 30 mice, each receiving 25 mg. of cinchophen plus 25 mg. of ascorbic acid plus 25 mg. of cysteine hydrochloride.

The chemicals were incorporated into a 5 per cent polyvinyl alcohol solution in such a way that 1 cc. of the resulting material contained the stated dose. A syringe provided with a blunted needle was employed for the administration of the solution.

The mortality rates in the four groups four and a half and twenty-four hours after administration are given in table 1.

This experiment was repeated with a 0.5 per cent tragacanth solution being used as the suspending vehicle. A fifth group, receiving 25 mg. of choline

TABLE 2.—*Effect of Certain Detoxicants of Cinchophen on the Mortality Rate of Cinchophen-Treated Mice (Repetition of Experiment in Table 1)*

Medication	Mice Dead	
	4.5 Hr.	24 Hr.
Cinchophen	23	27
Cinchophen + ascorbic acid	18	23
Cinchophen + cysteine hydrochloride	30	30
Cinchophen + ascorbic acid + cysteine hydrochloride	30	30
Cinchophen + choline dihydrogen citrate	30	30

dihydrogen citrate in addition to 25 mg. of cinchophen, was added. The mortality rates are given in table 2.

As these experiments suggested that ascorbic acid might exert a limited protective action against the acute toxicity of cinchophen, a third experiment was undertaken in which increasing doses of ascorbic acid (multiple weight equivalents of the standard dose of 25 mg.) were given. The suspending agent was again a 0.5 per cent tragacanth solution. There were 30 mice in each group. The experimental conditions observed and the results obtained are evident from table 3.

It was noted that the mice receiving the higher doses of ascorbic acid appeared to be more drowsy than those fed cinchophen only.

Since the possibility existed that some of the untoward effects recorded in the last experiment might be attributable to a severe osmotic action exerted by the considerable amounts of ascorbic acid introduced, an additional experiment was devised in which this factor was excluded as much as possible by using a freshly prepared sodium ascorbate solution in place of ascorbic acid. Cinchophen was suspended in a 5 per cent polyvinyl alcohol solution for the first two groups and in a 0.5 per cent tragacanth solution for the third group. The usual dose of 25 mg. of cinchophen was given to all mice, with 30 mice in each group. The experimental conditions observed and the results obtained are presented in table 4.

TABLE 3.—*Mortality Rates in Three Cinchophen-Treated Groups Given Increasing Doses of Ascorbic Acid*

Medication	Mice Dead at 24 Hr.
Cinchophen (control)	22
Cinchophen + 25 mg. ascorbic acid	22
Cinchophen + 50 mg. ascorbic acid	24
Cinchophen + 75 mg. ascorbic acid	22

From the total evidence recorded the conclusion was reached that none of various detoxicants (cysteine, ascorbic acid, choline), nor any mixture of them, exerted in acute experiments any significant and consistent protective action against a 50 per cent lethal dose of cinchophen given by mouth.

Chronic Experiments in Detoxication.—(*a*) Mice: These experiments were designed for the purpose of ascertaining the possible detoxicating power of ascorbic acid and methionine when moderately toxic doses of cinchophen were given over a period of several days or weeks, imitating in this respect more closely the conditions existing in the therapeutic use of the drug. Methionine was selected for testing because of the recent reports crediting this amino acid with beneficial properties when used in the treatment of hepatic degenerations caused by chlorinated hydrocarbons. A 0.5 per cent tragacanth solution was used as the suspending agent for cinchophen and as the solvent for the detoxicating chemicals. The experimental conditions and the mortality rates after two weeks of daily administration of the test substances are apparent in table 5.

TABLE 4.—*Mortality Rates in Two Cinchophen-Treated Groups in Which Sodium Ascorbic Was Substituted for Ascorbic Acid*

Medication	Mice Dead at 24 Hr.
Cinchophen in polyvinyl alcohol solution	18
Cinchophen + mg. ascorbic acid as sodium ascorbate in polyvinyl alcohol solution	27
Cinchophen + 25 mg. ascorbic acid as sodium ascorbate in tragacanth solution	30

The autopsies performed on many of these mice, some of which died during the course of the experiment and some of which were killed at the end of it, did not reveal any significant hepatic abnormalities. The conclusion drawn from this experiment was that the toxic effects of repeated administration of cinchophen

are not unequivocally mitigated by simultaneous medication with ascorbic acid or methionine.

(*b*) Rats: In view of the known species-specific variations existing in metabolization and in the detoxication of poisonous chemicals associated therewith, rats were employed in a second attempt directed at determining the possible protective power of ascorbic acid in cinchophen poisoning. These rats received, mixed with 5 Gm. of their stock diet, a daily dose of 60 mg. of cinchophen. This dose is approximately the maximal amount of cinchophen which rats will consume without forced feeding. Thirty rats weighing from 130 to 180 Gm. were fed 5 to 7 Gm. of this diet daily for six weeks. The daily offering of food was then increased to 7 to 10 Gm. but after two weeks it was reduced again to 5 to 7 Gm., as the rats did not consume the higher amounts offered.

TABLE 5.—*Effect of Detoxicants of Cinchophen on the Mortality Rate of Mice Receiving Cinchophen Over Considerable Periods*

Mice	Medication	Mice Dead at 2 Weeks
30	10 mg. of cinchophen	18
30	10 mg. of cinchophen + 10 mg. of ascorbic acid	22
30	10 mg. of cinchophen + 10 mg. of methionine	21
30	10 mg. of cinchophen + 20 mg. of ascorbic acid	16
30	10 mg. of cinchophen + 30 mg. of ascorbic acid	15

A second group of 30 rats was given the same diet fortified with 180 mg. of ascorbic acid per 5 Gm. of food-cinchophen mixture. There were the same variations in the amounts of food offered as in the first group. The weights of the rats of both groups either remained stationary or increased mildly during the experimental period of four months. There were no significant differences in fluctuation of weight between the two groups. Three rats of the cinchophen group and 2 rats of the cinchophen–ascorbic acid group died. The surviving rats were killed at the end of the experimental period. Autopsies did not reveal any significant abnormalities of the liver or of any other internal organ in these rats.

The conclusion was drawn that rats given the maximal tolerated dose of cinchophen with their food for four months exhibit, as the only sign of an injurious action, stunting of their growth, which is mainly caused by the reduction of their food intake. This effect is not prevented by simultaneous oral administration of ascorbic acid in amounts three times as large as those of cinchophen.

(*c*) Dogs. An experiment similar to the previous one was conducted on 10 dogs weighing between 9 and 11 Kg. Five dogs received daily 2.5 Gm. of cinchophen wrapped into fish preserve, while a second group of 5 dogs was given 2.5 Gm. of cinchophen together with 7.5 Gm. of ascorbic acid. A few days after the start of the experiment the dogs began to vomit following the ingestion of cinchophen; therefore, the daily amount of the drug was divided into two portions, one of which was given in the morning and the other at night in the stated fashion. The incidence of these reactions was thereby decreased but not entirely eliminated. Diarrhea, sometimes of a bloody character, was an early response to the medication in both groups. The appetite of the dogs receiving cinchophen only suffered after the first week of this treatment, while in the dogs given cinchophen-ascorbic acid this reaction made its appearance after the second week

had passed. With more prolonged feeding of the drugs, the dogs of both groups became increasingly weak and emaciated and were crouching in their cages. The dogs given cinchophen medication only died on the fifth, eighth, fifteenth, twentieth and twenty-fourth day, respectively, of treatment, while those given cinchophen plus ascorbic acid succumbed on the sixteenth, seventeenth, eighteenth, nineteenth and twenty-fourth day, respectively.

The rectal temperature was taken twice a day during the experimental period and did not reveal any significant fluctuations beyond the normal range. Blood examinations made during the first week did not show any appreciable changes in coagulation time, prothrombin time or sedimentation rate. In a few animals leukocytosis was present, accompanied by an increase of the lymphocytic elements and by moderate anemia, which was characterized by a reduction in the hemoglobin content and the number of erythrocytes. Determinations of the sugar and the uric acid content of the blood of the dogs of both groups, made on five occasions during the experimental period, revealed no significant changes in the blood sugar level and sometimes a mild lowering of the uric acid level. There were no differences in these reactions between the two groups. The postmortem examinations of the dogs revealed solitary or multiple ulcers of the stomach, usually located near the pylorus or on the pyloric ring. In a few instances ulcers were found at the lesser curvature. Some of the ulcers were large and deep. There was no demonstrable difference in the severity of these lesions between the two groups. The liver was without any gross abnormality in all animals. The histologic examination of the livers of the dogs of both groups showed moderate interstitial edema with scattered foci of degeneration of liver cells.

The observations made indicate that the addition of considerbale amounts of ascorbic acid, representing threefold equivalents by weight of the toxic dose of cinchophen administered, to the normal diet did not prevent the development of the usual toxic reactions and gastric ulcers elicited by cinchophen and did not delay appreciably the occurrence of the lethal response. The absence of any evidence of acute yellow atrophy of the liver in any of the dogs confirms similar findings previously made by other investigators.

COMMENT

The experiments reported show that cinchophen when given in excessive doses to rats kept on a diet low in vitamin K causes lengthening of the prothrombin time, and that, on the other hand, no such effect is obtainable in dogs fed a normal diet. Cinchophen thus behaves in this respect like the salicylates. It is improbable, therefore, that patients on a normal diet and given therapeutic doses of cinchophen by the generally adopted intermittent method of treatment will ever exhibit any lengthening of prothrombin time unless there develops severe hepatic degeneration.

The observations made in connection with the experiments on the influence of a low protein, low methionine diet on the toxicity of cinchophen, as well as those associated with the various detoxication experiments, provide additional weighty evidence militating against the

concept that cinchophen is a hepatotoxic substance in the ordinary sense of the word. None of the animals belonging to several species (mice, rats, dogs, monkeys) exhibited hepatic lesions comparable in any way to those present in acute yellow atrophy of the liver in man. While it is possible that such unfortunate and rare therapeutic accidents seen after the administration of cinchophen may have a chemoallergic origin, this is by no means the only possibilty. The evidence obtained in the present experiments indicates that malnutrition such as that caused by an insufficient intake of protein and particularly of methionine does not favor the development of severe and diffuse degenerative and necrotizing changes in the livers of animals given single and repeated toxic doses of cinchophen.

Attempts to protect animals given toxic or lethal single and repeated doses of cinchophen by the simultaneous administration of several natural detoxicating agents (ascorbic acid, cysteine hydrochloride, choline dihydrogen citrate, methionine) in doses representing one to threefold equivalents in weight of the cinchophen dose given failed completely. While the possibility remains that such agents may be effective in man, it is regarded as remote, especially as to the prevention of potential hepatic injury.

Finally, attention may be called to an observation made during the course of the experiments with a diet low in protein and methionine. It was found that in many of the rats the hairs of the nape, the ears and the snout showed porphyrin incrustations which were more marked and occurred earlier and more generally in the rats receiving the special diet together with cinchophen than in those fed the diet only. It was noted that these manifestations, usually found in connection with certain vitamin deficiencies (riboflavin, pantothenic acid), were not obviated when large amounts of these vitamins were added to the diet. Mention may be made in this connection of observations made by Bessey and Wolbach,[7] who found in rats kept on a diet deficient in lysine or tryptophan corneal lesions similar to those in animals with riboflavin deficiency, while Maun, Cahill and Davis [8] noted corresponding ocular changes in rats kept on a diet deficient in leucine. These findings suggest that there may exist a connection between deficiencies of certain vitamins and certain amino acids or their metabolic utilization, on one hand, and abnormalities of the prophyrin metabolism and corneal vascularization and ulceration, on the other.

7. Bessey, O. A., and Wolbach, S. B.: J. Exper. Med. **69**:1, 1939.
8. Maun, M. E.; Cahill, W. M., and Davis, R. M.: Arch. Path. **40**:173, 1945.

SUMMARY

Cinchophen given to rats kept on a diet low in vitamin K lengthens the prothrombin time, but it does not have this effect when administered to dogs fed a normal diet.

Rats receiving a diet low in protein and methionine and given, in addition, toxic doses of cinchophen do not show diffuse and severe degeneration of the liver, but exhibit porphyrin incrustations of the hairs of the nape, the ears and the snout similar to those observed in riboflavin and pantothenic acid deficiency.

Monkeys given toxic doses of cinchophen by mouth do not have as a result acute yellow atrophy of the liver or gastric ulcers.

Mice, rats and dogs given single and repeated toxic and lethal doses of cinchophen by mouth and simultaneously various detoxicants (ascorbic acid, cysteine, methionine, choline) do not show any appreciable mitigation of the toxic symptoms or delay of or protection against the lethal effect.

HUMAN STRONGYLOIDIASIS WITH INTERNAL AUTOINFECTION

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DESPITE the frequency of strongyloidiasis in many tropical and subtropical countries,[1] only little can be found in the literature concerning the histologic aspects of this disease as it occurs in man and even less concerning the internal autoinfection with Strongyloides stercoralis. Thus Faust and De Groat,[2] reporting internal autoinfection in a case of human strongyloidiasis, list not more than four papers containing reports of 5 analogous cases. This scarcity of case reports and exact descriptions of the histologic changes can be easily explained by the generally benign course of the disease, by the difficulty of finding the parasites at gross examination and by the fact that routine microscopic examination of the intestines is only rarely performed and its value often rendered illusory by extensive autolysis and putrefaction.

It can therefore not be contended that the histologic picture of human strongyloidiasis with or without internal autoinfection is well established, and thus the description of an additional case seems worth while, especially since the conditions under which the autopsy was performed were very favorable for the subsequent histologic examination.

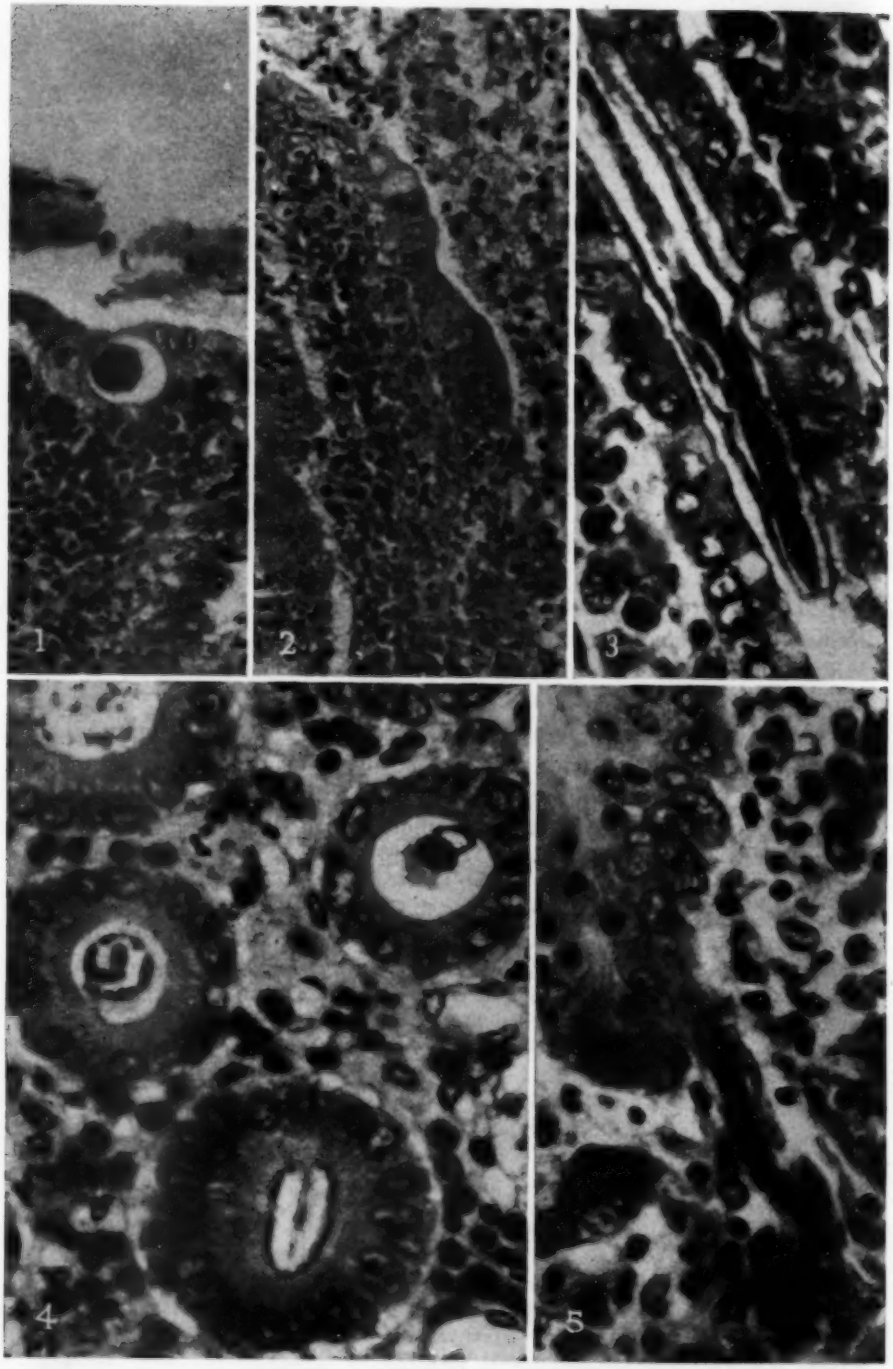
REPORT OF A CASE

A Negro 46 years old was admitted in a bad condition to the hospital for patients with mental disorders. Clinically he presented the classic picture of dementia paralytica with megalomania. The Nonne-Apelt and Pandy reactions of the cerebrospinal fluid were positive, and the colloidal gold curve was of the paretic type. The glucose content was 110 mg. per hundred cubic centimeters. A few weeks before his death the patient became bedridden. During the last two days he had frequent thin, evil-smelling stools, macroscopically without blood and mucus. He died nine months after admission.

Autopsy was performed forty minutes after death. The internal organs were atrophic. The mucous membranes of the colon and the ileum were somewhat

1. (*a*) Brumpt, E.: Précis de parasitologie, ed. 5, Paris, Masson & Cie, 1936, p. 889. (*b*) Faust, E. C.: Human Helminthology, ed. 2, Philadelphia, Lea & Febiger, 1939, p. 391. (*c*) Belding, D. L.: Textbook of Clinical Parasitology, New York, D. Appleton-Century Company, Inc., 1942, p. 271. (*d*) Strong, R. P.: Stitt's Diagnosis, Prevention and Treatment of Tropical Diseases, ed. 7, Philadelphia, The Blakiston Company, 1944, p. 1278.

2. Faust, E. C., and De Groat, A.: Am. J. Trop. Med. **20**:359, 1940.



Figures 1 to 5

(See legend on opposite page)

swollen and hyperemic; no ulcers were observed. There was some thickening of the wall of the duodenum. The mesenteric lymph nodes were swollen. Examination of the brain was impossible.

Microscopic Examination.—The duodenum was fixed in Bouin's fluid; from the colon and other organs blocks were fixed either in Orth's or in Bouin's fluid. Péterfi's methyl benzoate–celloidin method for paraffin embedding was used; for the blocks fixed in Orth's fluid the celloidin (a concentrated preparation of pyroxylin) in the methylbenzoate medium was increased to 4 per cent. The sections were stained with hematoxylin-azophloxine, orange-eosin-toluidine blue, phloxine–methylene blue, gallamine blue, azocarmine–aniline blue, phosphotungstic acid–hematoxylin, Gram's stain and mucicarmine.

(*a*) Duodenum: The adult worms were not numerous. They were found partly in the stroma of the villi or in the intervillar spaces. Rarely they were lying in the deeper parts of the crypts of Lieberkühn. The cuticula of the worms showed fine cross striation, which was especially well visible with Gram's stain, as this stained the cuticula pale violet. The cross striation consisted of alternating lighter and darker staining bands, the latter also showing faint longitudinal striation. Where the adult worms were lying in the stroma of the villi there was surprisingly little reaction. Only in one place a small giant cell was observed in close contact with a worm, and the stroma of the villi containing adult worms was infiltrated by plasma cells, as was also the stroma of the noninfected villi. Eosinophilic leukocytes were seen in moderate numbers, but there was no pronounced eosinophilia. The adult worms were never surrounded by epithelial sheaths, aside of course from those parts of the worms which occupied an intraepithelial position.

The eggs and the earlier developmental stages of the larvae were found exclusively in the epithelium. They were often lying in groups. They were not confined to the epithelium of the crypts but were frequently observed in the epithelium of the villi, sometimes even on the top of a villus. The eggs or the younger larval stages were completely surrounded by epithelial cells, which sometimes were greatly thinned and contained flattened nuclei but remained recognizable as such.

The older larvae seemed to have migrated from their intraepithelial position into the crypts or into the intestinal lumen, where they were found in great numbers. When in the lumen, they were never surrounded by epithelial sheaths; it should, however, be noted that during the final stages of the migration from the "epithelial chamber" the epithelial cells could be greatly drawn out and thinned and still adhered to worms that were already lying in the lumen. Intraepithelial cavities through which adult worms had migrated, or which had con-

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EXPLANATION OF FIGURES 1 TO 5

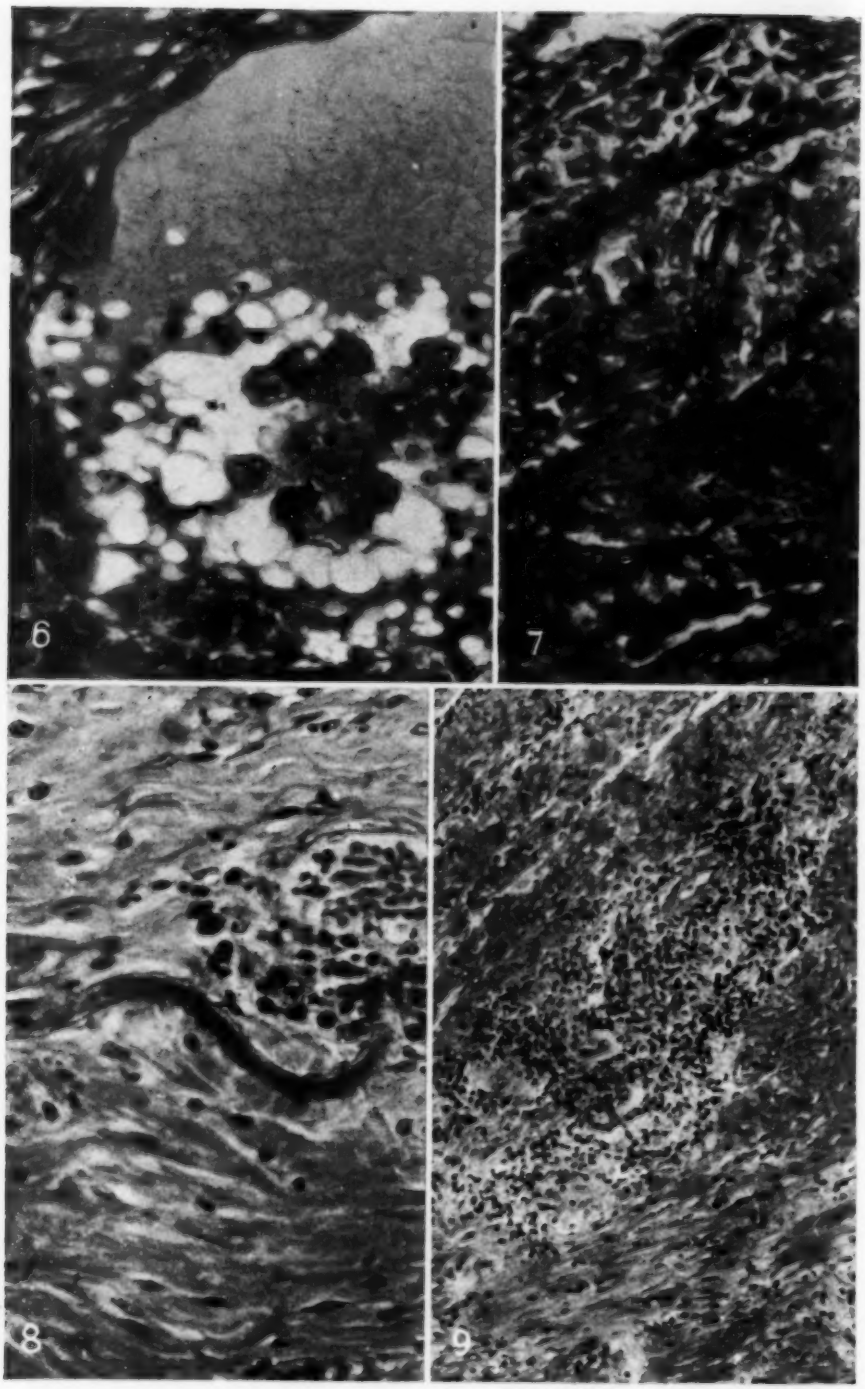
Fig. 1.—Tip of a duodenal villus with an egg of Strongyloides stercoralis in an intraepithelial position. Hematoxylin-azophloxine; × 315.

Fig. 2.—Acute inflammation of the duodenum. The epithelium contains many leukocytes, but there is no desquamation. Hematoxylin-azophloxine; × 315.

Fig. 3.—Larva in the lumen of a crypt of Lieberkühn in the colon. Phosphotungstic acid–hematoxylin; × 645.

Fig. 4.—Cross sections of crypts of the colon containing larvae. Phosphotungstic acid–hematoxylin; × 645.

Fig. 5.—Larva emerging from a crypt in the colon. The epithelium is hyperplastic and shows several mitotic divisions. Hematoxylin-azophloxine; × 500.



Figures 6 to 9

(See legend on opposite page)

tained larvae and were now empty, were relatively rare; they contained sometimes large gram-positive and gram-negative bacteria and gram-positive cocci, whose nature could not be determined more exactly.

The epithelium of the villi was mostly intact, and in the proximal parts of the duodenum small islets of gastric epithelium could be recognized. Mitotic divisions of epithelial cells were not confined to the crypts but were seen also in the tips of the villi. Sometimes the impression was gained that in places the epithelium had become multilayered.

In many places, in the crypts and near or on the tips of the villi there were marked inflammatory changes, only rarely combined with fibrinoid necrosis of the tips of the villi. When there was inflammation in the deeper portions of the mucosa, there were nearly always larvae, together with bacteria and leukocytes, in the lumens of the crypts. Many leukocytes were seen migrating through the epithelium, which on the tips of the villi had sometimes degenerated or become necrotic. There was neither desquamation of epithelial cells nor fibrosis of the mucous membrane resulting from previous inflammation.

Brunner's glands were strongly developed and formed large accumulations of glandular tissue. Between the tubules of the glands there were a few plasma cells and eosinophilic leukocytes. In a few instances embryonated eggs were found in the epithelium or a larva in the lumen of one of these glands. There was a moderate number of mitotic divisions in the glandular epithelium. In many places the muscularis mucosae was poorly developed or completely absent.

(*b*) Colon: The surface epithelium was practically everywhere intact and only in a few places could small ulcers be seen microscopically. The epithelium of the crypts showed numerous mitotic divisions; it contained a moderate number of enterochromaffine cells and an occasional Paneth cell.

The larvae, the majority transformed into filariform larvae, invaded the intestinal wall by first entering the lumens of the crypts of Lieberkühn, as could be demonstrated in longitudinal and cross sections of the crypts, and by subsequently migrating through the epithelium, especially of the deeper portions of the crypts, with invasion of the stroma. It should be noted that this happened quite independently of the microscopically small ulcers. The impression was gained that during this migration the few rhabditiform larvae which had entered the crypts also transformed into filariform larvae. Where the larvae migrated through the epithelium, the epithelial cells appeared to be very large, with nuclei containing large nucleoli; in one place there were three or four mitoses just adjacent to the place of penetration. There were a few cystic dilated crypts containing leukocytes; from one of these crypts a larva was seen penetrating into the mucosal stroma.

The more superficial part of the mucosal stroma was in general densely infiltrated by plasma cells and by varying numbers of eosinophilic leukocytes.

EXPLANATION OF FIGURES 6 TO 9

Fig. 6.—Dilated lymph vessel in the submucosa of the colon. Large histiocytes surround part of a necrotic larva. Hematoxylin-azophloxine; × 315.

Fig. 7.—Larvae in partially obliterated lymph vessel of the colon. Azan stain; × 315.

Fig. 8.—Larva in the submucosa of the colon; the worm is partially degenerated. There is an accumulation of eosinophilic leukocytes. Hematoxylin-azophloxine; × 315.

Fig. 9.—Granulomatous inflammation in the muscular coats of the colon. Hematoxylin-azophloxine; × 150.

In the deeper layers, where often many larvae were present, the reaction provoked by the worms varied between wide limits. Giant cells were present in moderate numbers; they were of the smaller type; often worms, which by the strong eosinophilia and smudginess of their cuticula have perhaps to be considered as degenerating, were surrounded by large histiocytes, whereas a few others did not seem to have provoked any definite reaction. Some larvae were lying in small abscesses. Eosinophilic leukocytes were present in small numbers, sometimes lying together in small groups.

In the submucosa the lymphatics showed important but varying alterations. They all presented a more or less marked dilatation, especially the parts of the lymphatics which were lying close to the muscularis mucosae. Many contained large numbers of large and small lymphocytes and degenerating neutrophilic leukocytes; in others many eosinophilic leukocytes or erythrocytes were found. The presence of the larvae in the lumens of the lymphatics provoked reactions which varied in extent and type. In a few instances the vessels were filled with a fibrinoid substance, atypical giant cells, few epithelioid cells and some degenerating leukocytes, between which the remains of a dead larva could be detected only with difficulty. In other lymphatics intact larvae were observed which had provoked no reaction at all. Most frequently, however, the lymphatics in which there were larvae contained numerous large, rounded, sometimes finely vacuolated phagocytes with rounded to oval nuclei containing dustlike chromatin and one or two small but distinct nucleoli. Their protoplasm was faintly basophilic and contained sometimes phagocytosed cell rests. These cells occurred as isolated elements or were arranged in groups or solid sheets; they were accompanied by lymphocytes of different size and by a few leukocytes. They surrounded and sometimes completely encapsulated the larvae present in the lymphatics. In other vessels a kind of granulation tissue, composed of large cells which could be best described as forms intermediate between histiocytes and fibrocytes, with branching fibers, staining with aniline blue, between them, had encapsulated the worms and blocked the vessels. In this granulation tissue giant cells or eosinophilic leukocytes were only rarely seen. Sometimes a fibrinoid substance surrounded the worms or was found between the cells. There were no mitoses.

In a few instances larvae were found in small veins.

It was difficult to determine whether the larvae were still intact at the moment of fixation. Often it was observed that the cuticula, which in probably intact larvae was a thin membrane, staining red with phloxine–methylene blue or with orange-eosin–toluidine blue, became irregularly thickened and smudgy in larvae encapsulated by histiocytes, although the parenchymal cells of the worms did not show any changes. When the nuclei of these cells showed chromatolysis and the worm stained diffusely with eosin, it could of course be considered as dead.

In the connective tissue of the submucosa a few intact larvae were found. The majority, however, appeared to have been damaged and had provoked severe reactions. Their cuticulas showed the irregular thickening just mentioned, and often short bands of a fibrinoid substance seemed to radiate from the cuticular surface. Some larvae were surrounded by neutrophilic polymorphonuclear leukocytes; others, however, were surrounded by pale-staining histiocytes with relatively large nuclei, which often formed small giant cells by coalescence, by lymphocytes and plasma cells and especially by large numbers of eosinophilic leukocytes. Together these cells formed what can be designated as eosinophilic granulomas. It also happened that a part of a larva appeared to be intact and the rest degenerating and surrounded by leukocytes or histiocytes or both.

When there were a great number of worms, the submucosa was sometimes studded with small eosinophilic granulomas. Many larvae had penetrated into the muscular coats and into the subserous connective tissue. There were many worms in the intermuscular connective tissue septums, containing the blood vessels and nerves. These septums had been much broadened, and the connective tissue had been replaced to a large extent by granulation tissue containing large numbers of eosinophilic leukocytes. Other larvae had penetrated between the muscle fibers, provoking acute or granulomatous reactions in the edematous muscular tissue. Here several worms showed far advanced disintegration, their bodies being diffusely eosinophilic. In the subserosa and in the mesocolon and the mesosigmoid there were many granulomas, with small giant cells containing few but

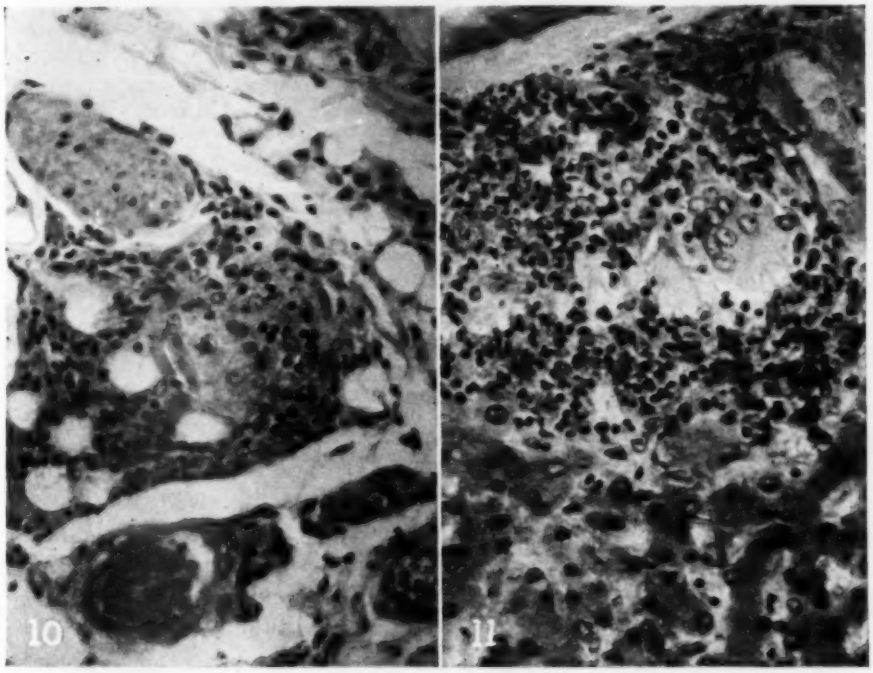


Fig. 10.—Mesocolon showing an eosinophilic granuloma with pale-staining histiocytes, containing a larva, which is seen in cross section. Hematoxylin-azophloxine; × 315.

Fig. 11.—Subcapsular eosinophilic granuloma in the liver. Hematoxylin-azophloxine; × 315.

large nuclei and pale-staining protoplasm and many eosinophilic leukocytes, enclosing larvae. However, there were also intact larvae lying free between the connective tissue fibers or sometimes in lymph vessels, without having provoked any reaction.

(*c*) Small Intestine: One block of small intestine was examined. There were a few isolated eggs in the epithelium of the villi; the mucosal stroma was hyperemic and infiltrated by plasma cells. Pathologic changes of the type which

occurred in the colon were observed here, though on a lesser scale. However, the number of intact larvae present in the submucosa without having provoked any reaction was much greater.

(*d*) Mesenteric Lymph Nodes: The cortical follicles were atrophic; the medullary cords were well developed; they contained many large lymphocytes; these cells occurred also in great numbers in the intermediate sinuses. There was definite hypereosinophilia, the eosinophilic leukocytes being irregularly scattered through the nodes. In one node a partially disintegrated filariform larva was seen; one part stained diffusely with eosin; the other part contained well preserved nuclei. The worm was surrounded by a small number of eosinophilic and neutrophilic leukocytes and a few histiocytes.

(*e*) Liver: The centers of the small lobuli disclosed hyperemia and fatty infiltration, as well as atrophy and pigmentation of the liver cells. Mitoses in liver cells were fairly numerous. There were almost no eosinophilic leukocytes in the sinusoids.

Filariform larvae were observed just below the capsule and sometimes deep in the liver in the periportal spaces. All larvae were enclosed in small eosinophilic granulomas.

(*f*) Stomach: Only one block, taken from the border region of corpus and antrum, was examined. It showed some chronic inflammation with marked proliferation of the epithelium.

(*g*) Pancreas: There was marked atrophy of the acinous tissue and of the islets. Several small intralobular ducts were dilated and contained inspissated secretion.

(*h*) Other organs: The heart and the adrenal glands did not show anything worth mentioning.

COMMENT

The case described presents several interesting features. In the first place the almost complete absence of ulceration in the intestine should be noted. The few microscopic ulcers that were seen were probably caused by the presence of larvae in the mucous membrane and represented small abscesses that had opened into the intestinal lumen. The invasion of the intestinal wall was quite independent of this ulceration. It could be demonstrated that the larvae entered the crypts of Lieberkühn and from there invaded the mucous membrane. Before or during this invasion most larvae transformed into filariform larvae. In the mucous membrane they provoked inflammatory reactions of different type and intensity. The larvae seen in the colon were probably coming from the small intestine, especially from the duodenum, the only place where adult worms and eggs in large numbers were found.

After penetrating into the submucosa and into the other layers of the intestinal wall, the larvae provoked the formation of numerous small granulomas composed of histiocytes, small giant cells, plasma cells and large numbers of eosinophilic leukocytes. These eosinophilic granulomas were also found in the mesocolon and in the liver. Sometimes more acute reactions were observed, especially in the muscular coats.

The alterations of the lymphatics, especially in the submucosa, were conspicuous. Apart from dilatation of these vessels and the presence in the lumens of many large lymphocytes and eosinophilic leukocytes, there was granulomatous endolymphangitis, provoked by the presence of the larvae in the lumens. This granulomatous inflammation led to encapsulation of the larvae and often to more or less complete obliteration of the involved lumens with consequent dilatation of the nonobliterated peripheral parts of the vessels. In contrast to filarial endolymphangitis, in which oval or more elongated epithelioid cells predominate,[3] here large, rounded, finely vacuolated histiocytes were more frequent; in what must be considered as more advanced stages, when fibers staining with aniline blue developed between the cells, the difference from filarial endolymphangitis became less outspoken, though, in contrast to filariasis, there were always worms inside the granulomas.

In several instances intact larvae were found in venules of the submucosa or in intact lymph vessels. In this way the lymph nodes and the periportal spaces in the liver probably were infected. The larvae found just below the capsule of the liver may perhaps have migrated from the peritoneal cavity, after migrating through the intestinal wall, though this cannot be proved.

It is not difficult to understand that the inflammation of the wall of the bowel, together with the alterations of the lymphatics, may have caused marked functional disturbances; unfortunately the patient was not closely observed and apart from the diarrhea nothing special was noted.

Disintegration of the larvae was observed; it was difficult to detect the early stages of this process and to determine whether or not a worm was still intact. Leukocytic invasion of the larvae was only rarely seen, though it occurs in later stages of disintegration, as was observed in another case.

In the liver and in the lymph nodes only slight changes were noticed. In the liver the worms were lying inside granulomas. I do not think that the fact that numerous mitoses were seen in the liver cells should be attributed to the presence of larvae in that organ.

In the lymph nodes no granulomas were found; the only larva found here was surrounded by neutrophilic and eosinophilic leukocytes and a few histiocytes.

Faust and De Groat [2] observed in their case that once the larvae had broken through the barrier of the muscularis mucosae the damage to the tissues was slight, and no attempt other than moderate eosinophilic infiltration was made to stay the migration of the filariform larvae. This is in marked contrast to my case, in which there were many eosinophilic

3. Hartz, P. H.: Am. J. Clin. Path. **14**:34, 1944.

granulomas and conspicuous granulomatous endolymphangitis. This great difference in cellular reaction against the same parasite in 2 persons must perhaps be explained by difference in reactivity of the hosts; the immunity which can be obtained experimentally against Strongyloides points in this direction.[4]

In the duodenum the eggs were found exclusively in the epithelium and never in the stroma. There was proliferation of the epithelial cells, proved by the occurrence of mitotic divisions, even on the tips of the villi. No desquamation of epithelial cells was observed, and it is believed that the epithelial desquamation described by Strong [1d] in the duodenum in strongyloidiasis must be attributed to postmortem changes.

The eggs and the young larvae probably exert a stimulating effect on the epithelium, thus causing proliferation. In this way, also by the pressure exerted by the growing larvae, the small "incubation chambers" with partially flattened cells are formed, from which later the rhabditiform larvae escape. I cannot agree with Faust [5] when he considers this a defensive measure of the body acting against the parasites. Such active encapsulation of parasites by true epithelium would be an exceptional reaction from the standpoint of mammalian pathology, and in my opinion the opposite is the case; the larvae are protected by the epithelium during their development.

It is logical that when an adult worm penetrates through the epithelium the epithelial cells are pushed aside and compressed, just as can be observed on a larger scale in a freshly fixed colon containing trichocephalus. To designate such compressed epithelium as an adventitious sheath is certainly an exaggeration. It is also possible that these sheaths, as depicted by Faust,[5] are artefacts produced by ungentle handling of the tissues during autopsy or preparation. As several of Faust's photomicrographs [6] show extensive damage of the tissues, this possibility cannot be excluded.

In several places there was acute inflammation of the duodenum of a type not commonly observed, which was probably caused indirectly by the presence of the larvae and adult worms, since several cavities formed by the worms or eggs contained large numbers of micro-organisms.

That a few eggs and larvae were found in Brunner's glands can be explained by the fact that in duodenums with strongly developed or hyperplastic Brunner's glands the muscularis mucosae, which otherwise seems to serve as an effective barrier against the penetration of the

4. Culbertson, J. T.: Immunity Against Animal Parasites, New York, Columbia University Press, 1941, p. 211.

5. Faust, E. C.: Arch. Path. **19**:769, 1935.

6. Faust,[5] figs. 5, 8, 12A and 15.

worms, may be partially absent. It is not sure whether or not this hyperplasia and the mitotic activity of Brunner's glands must be attributed to the presence of the worms, as they can be observed in different circumstances.[7]

SUMMARY

A Negro who died of dementia paralytica was found to be infected with Strongyloides stercoralis. The adult worms were found exclusively in the duodenum. The larvae invaded the small intestine and especially the colon by entering the crypts of Lieberkühn and penetrating through the epithelium. The invasion was independent of ulceration. The larvae generally were transformed into the filariform type. After migrating through the mucous membrane, where they provoked inflammatory reactions of different type and intensity, they invaded the deeper layers of the intestinal wall, where they caused the formation of numerous eosinophilic granulomas which encapsulated the larvae and which were also found in the mesocolon and in the liver. A few intact larvae were noted in lymph vessels, in small veins and in the connective tissue of the submucosa and the subserosa of the small intestine and colon. In many lymphatics the larvae provoked granulomatous endolymphangitis often with occlusion of the lumens and dilatation of the peripheral parts of the vessels. It could be distinguished from filarial lymphangitis. In the mesenteric lymph nodes there was moderate diffuse eosinophilia but no granulomatous reaction; the granulomatous reaction was present again in the liver. It must be considered probable that the extensive alterations of the colon caused important functional disturbances.

7. Hartz, P. H., and van der Sar, A.: Am. J. Path. **20**:931, 1944.

TROPICAL ULCER IN GUATEMALA

Pathologic, Bacteriologic, Mycologic and Clinical Aspects

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TROPICAL ulcer affects great numbers of the population in Central American countries. Because of the chronicity of the lesion, the disease is responsible for a large percentage of the hospitalizations in those countries. The plan of procedure devised for this study encompassed the selection of patients with early, "untreated" lesions as far as possible, in order to avoid interference with subsequent pathologic and microbial studies. It was soon found, however, that most patients had attempted some form of self medication. A few with totally untreated lesions were found, and the remainder were selected as far as possible from those whose therapy appeared least likely to interfere with our studies. Observations on a total of 24 patients are presented here. The history of the lesion was secured in the patient's words. In the physical examination the appearance of the ulcer was recorded, the skin was surveyed for abnormalities, a search was made for predisposing factors such as varicose veins, arteriosclerosis, diabetes mellitus or the sickling trait of erythrocytes, the oral temperatures, the pulse rates and the respiratory rates were recorded, the thorax and the abdomen were observed by the usual methods, and the spleen and the superficial lymphatic system were tested by palpation, particular attention being paid to the lymph nodes regional to the ulcer. Specimens of the urine and the stools were examined, serologic tests for syphilis and complete blood counts were made and sedimentation rates observed for most of the patients. A dietary history was obtained from 23 of the 24 patients, who were asked about the foods taken at their usual meals, and the data were recorded qualitatively. If one considers the rather monotonous diets of our patients, the method used was of fair accuracy. In each instance the lesion was then photographed. Whenever possible with those selected for biopsy, ulcer medication, if any, was discontinued for a few days prior to the

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study, and the ulcer was covered with sterile gauze squares soaked in isotonic solution of sodium chloride. Iodine-alcohol disinfection of the cutaneous borders before biopsy was followed by intracutaneous injection of a 1 per cent solution of procaine hydrochloride sufficiently distal to the edge of the ulcer to avoid tissue artefacts on subsequent microsection. Immediately prior to the aseptic removal of a rectangle of ulcer consisting of granulation tissue, border of ulcer and a segment of skin, the area was frozen lightly with ethyl chloride spray (fig. 1 *A*). In the earlier cases of the series swabs were passed over the ulcer surfaces to secure material for microbial analysis prior to biopsy. In a few other cases the aseptically removed tissue was impressed by contact on suitable mediums for the culture of the organisms present. In the remainder (over half of the cases) the tissue was divided in half; one portion was ground in sterile quartz sand for microbial analysis, and multiple fragments were planted on Sabouraud's medium for mycologic study; the other half of the specimen immediately after removal was fixed in a 4 per cent aqueous solution of formaldehyde, then embedded in paraffin and sectioned serially. Drawings and photographs made during these procedures supplied the necessary data for gross and microscopic correlations in later studies.

Most of our patients were hospitalized. In all instances the patient's residence at the time of the onset of the ulcer was noted, and the elevation above sea level was determined from maps later. The patients tolerated biopsy well. There was no tendency for the operative site to show other than uneventful healing. In fact, in a few early cases there was rapid involution of the area and of the whole ulcer, healing taking place in several days to a few weeks by formation of a puckered scar. This should not be taken as suggested therapy from our evidence alone, since there were a number of cases in which tropical ulcer in an early stage tended to undergo spontaneous involution on rest in bed alone.

CLINICAL ASPECTS

Affecting universally the lower half of the lower leg without predilection for the right as against the left leg, there may be an ulcer varying in size from a fraction of a centimeter to several centimeters in diameter. In the earliest stage seen, in cases of about two weeks' duration, the ulcer tends to be round or oval in outline, with a definitely raised, rolled narrow border of skin enclosing a bright red, finely nodular granulation tissue. The edge is usually slightly undermined, and small deeper pockets of underdipping may be encountered. The edge is epithelized and is directly continuous with unaltered surrounding skin. The surface of the ulcer at this stage is free of exudate; on pressure it may exude a small amount of sanguineous fluid (fig. 1 *B*). At this stage there is little or no odor. With pro-

gression of the lesion for months, usually accompanied by slow involution, there is a gradual change of color of the granulation tissue surface of the ulcer from bright red to pale pink, along with formation of coarse, irregular nodules. The depressions between nodules may contain small pools of pale yellow to greenish yellow exudate, sometimes putrid. In no instance were adherent or diphtheritic membranes encountered. The edge of the ulcer tends to become flatter with time and assumes a whitish opacity, finally becoming silvery and scarlike. It is at some time during this period that induration, discoloration and flattening of the skin surrounding the edge of the ulcer take place (fig. 1 *C*). The color changes in the surrounding skin, usually accompanied by mild edema, are pink to red to purple in the earlier stages, becoming brown to bronze with subsequent induration. At the same time the affected skin loses its linear markings, and the hair follicles disappear. Eventually there is formed a flat, evenly depressed, indurated surface (fig. 1 *D*), which is usually hyperpigmented. Apparently by varying rates of spread and involution, the ulcer assumes an irregular outline. After a duration of many months or years the lesion eventually shows large zones of ulceration lying in the midst of extensive areas of induration and fixation, even to the point of involving the largest part of the lower leg, but rarely exposing underlying muscle or bone. At some time during this phase there appears around the edge of the ulcer an area of depigmentation terminating as an opaque white, irregular zone which gradually extends peripherally (fig. 1 *E*). At any stage one might see reepithelization varying in form from buds to bands which tend to grow across the ulcer surface, usually ending abortively. Tenderness, as a rule, is minimal, and in the later stages the skin surrounding the ulcer may be almost anesthetic.

Healing may take place at any of the earlier stages, rarely in the middle stages and probably not at all in the last described. Such healing is frequently spontaneous or occurs in spite of the remedies usually applied. As these areas heal a puckered thin scar is formed. Around the scar is a zone which gradually achieves or has achieved a deep bronze pigmentation, which lasts for years if not indefinitely.

All of the cases studied or surveyed originated in the lowlands (from sea level up to 1,000 feet [300 meters] above sea level), and comprised members of the age groups from the second through the seventh decade. The population studied is predominantly American Indian and Indian-Caucasian. Consequently most of the patients in our series are of that physical type (table 1). In our surveys, however, all types living in the lowlands were found affected: Indian, Caucasian and Negro. No significant difference of incidence was noted between males and females in hospitals or in the field. Occupation appeared to play no predisposing role in the origin of the ulcer.

The medical history was our only approach to an evaluation of the origin of tropical ulcer, with all the vagueness and paucity of detail that the method implies. Fly, mosquito, or other insect bites were indicated as the primary injury at the ulcer site in 6 cases; in 8 cases there was mention of trauma; in 9 cases only an initial "blister," "boil"

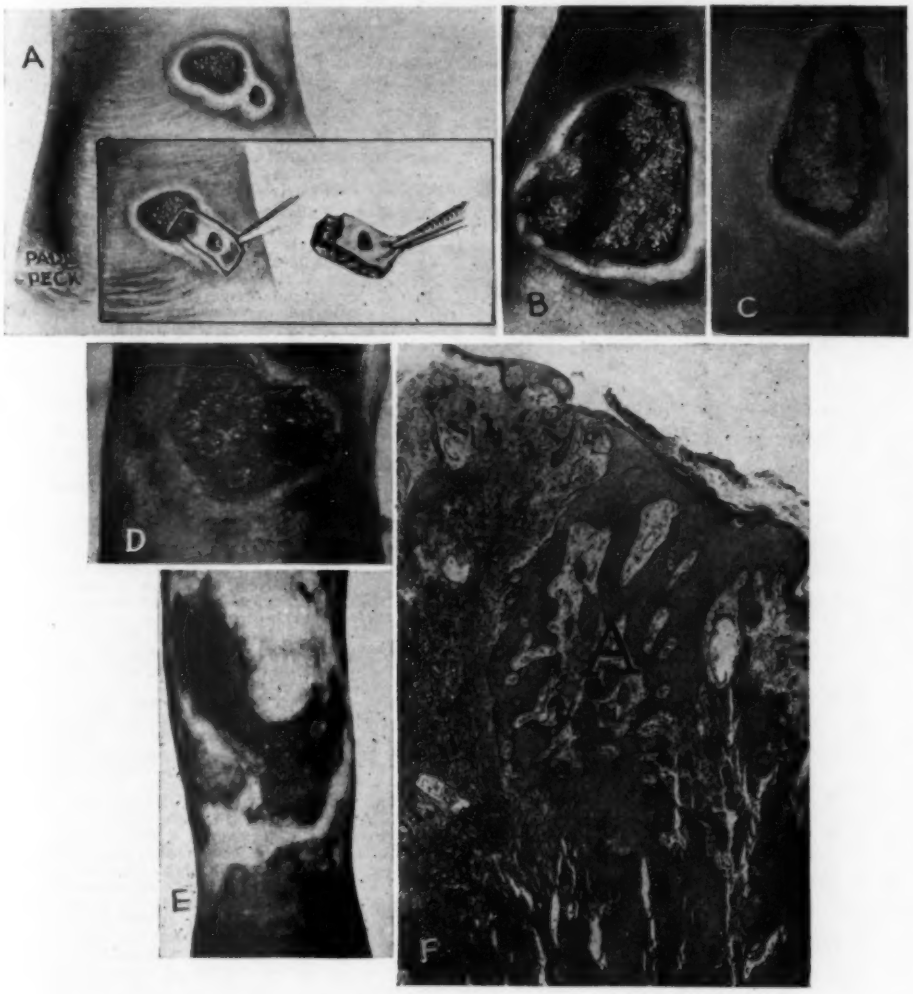

Fig. 1.—*A*, drawing showing the technic of biopsy. The patient (no. 1) presents a satellite ulcer, which was removed with the main biopsy specimen en bloc. *B*, tropical ulcer of fourteen days' duration (patient 17). *C*, tropical ulcer of two years' duration (patient 15). *D*, tropical ulcer of eleven months' duration (patient 16). *E*, tropical ulcer of more than one year's duration (patient 20). *F*, margin of ulcer of patient 17, of fourteen days' duration, showing moderate pseudoepitheliomatous hyperplasia (*A*); × 25. (Army Medical Museum negative, no. 89821.)

or "pimple" was reported; in 1 case the remembered details were too vague, or the patient did not know of any antecedent factors. Obviously, no class of injuries was common to these cases at the onset of ulceration.

In all but 1 case the temperature, the pulse rate and the respiratory rate were normal either at the time of the examination or at the time

TABLE 1.—*Data on Patients with Tropical Ulcer*

Patient	Age	Sex	Race	Duration of Lesion	Size of Lesion, Mm.	Bacterial Flora
1	14	F	Indian*	2-3 mo.	10 × 13	No isolations; gram-positive cocci
2	26	F	Negro	1 mo.	34 × 21	No isolations; gram-positive cocci
3	19	F	Negro	1 yr.	22 × 25	Staph. aureus; Micrococcus luteus (?)
4	17	M	Indian	4 mo.	78 × 51	Staph. aureus
5	42	M	Indian	2 mo.	68 × 47	Unidentified gram-positive micrococci; Micrococcus aurantiacus (?)
6	33	M	Indian	3 wk.	9 × 12	Isolations of gram-positive cocci lost before final identification
7	37	M	Indian	3 mo.	13 × 11	Gram-positive cocci (some probably streptococci, others staphylococci—not further identified)
8	20	F	Indian	20 days	13 × 13	Staph. aureus
9	42	F	Indian	1 mo.	10 × 8	Staph. aureus
11	16	F	Indian	2 mo.	25 × 28	Staph. aureus
14	28	M	Indian	5 wk.	51 × 20	Staph. albus; Staph. citreus
15	28	F	Indian	2 yr.	21 × 39	Staph. aureus; Staph. epidermidis; undetermined bacillus (gram-negative nonmotile); a streptococcus of undetermined species
16	18	F	Indian	11 mo.	31 × 33	Streptococcus, species undetermined; Staph. aureus
17	21	M	Indian	14 days	28 × 31	Gram-positive diplococci, not further identified; a gamma hemolytic anaerobic streptococcus, species undetermined
19	17	F	Indian	15 days	16 × 22	Gram-positive bacillus, not further identified; Str. durans (?); Staph. aureus; unidentified gram-positive cocci, and gram-positive bacilli
21	17	F	Indian	3 mo.	47 × 56	Staph. aureus; Proteus vulgaris (?); Micrococcus luteus (?); unidentified gram-positive bacilli
22	11	F	Indian	2 mo.	7 × 5	Staph. aureus; an anaerobic streptococcus of undetermined species
23	25	F	Indian	Undetermined	28 × 18	Proteus vulgaris; Micrococcus luteus (?); a diphtheroid
24	17	F	Indian	3 mo.	19 × 24	Bacterium zopfii; a gram-positive bacillus, not further identified; an anaerobic, hemolytic streptococcus of undetermined species
25	14	F	Indian	3 mo.	39 × 9	Diplococcus pneumoniae; gram-negative bacilli; gram-positive bacilli, not further identified
27	56	M	Indian	10 yr.	94 × 34	Staph. albus; Str. haemolyticus; Staph. aureus

* The term is used to denote all those showing the Central American Indian type of features and general physique. Many of the patients included in this term represented admixtures of Indian and Caucasian types.

the hospital record was made or at both times. Blood pressure readings were taken from the arm and from the legs by the usual auscultatory method. In 6 cases the blood pressures of the legs were at least 10 mm. of mercury higher than those of the arm (i. e., popliteal artery

vs. brachial artery) in the prone and the supine position, respectively. Such differences are within physiologic limits.[1] The highest blood pressure recorded was that of a 17 year old girl (patient 21), being 150 systolic and 65 diastolic. The other patients showed no significant deviations from the normal. No cases of tropical ulcer associated with varicose veins of the legs were included in this series.

No significant abnormalities were noted in the skin of the extremities, particularly in that of the comparable area of the leg not ulcerated, or in the skin of any other part of the body. No lesions were found on physical examination of the thorax of any patient included in this series.

The popliteal lymph nodes were not palpable in any patient studied or in those surveyed, with duration of the lesion ranging from a few weeks to several years. The inguinal lymph nodes were found sometimes to be slightly enlarged but never tender or painful; the nodes on the side of the lesion were no larger than those on the unaffected side. There was no sign of lymphangitis even in the patients with the earliest or most actively progressive ulceration.

The data fror the clinical laboratory may be summarized as follows: No abnormalities were encountered in urinalyses. Thick blood smears (usually but one smear per patient) revealed no malarial parasites, although enlargement of the spleen was found in patients 2, 5, 7, 11, 20 and 25, and patient 18 gave a history of splenomegaly with involution. Hemoglobin values varied considerably, as did estimated numbers of red blood cells per cubic millimeter, the usual finding being moderate hypochromic, normocytic anemia. White blood cell counts varied from about 4,000 (leukopenia) to over 18,000 (leukocytosis) per cubic millimeter, with most of the values within normal limits. There was no instance of polymorphonuclear leukocytosis. A fairly constant finding was eosinophilia, the percentage of eosinophils not exceeding 23. In this connection it should be noted that the patients of our series showed a high incidence of intestinal parasitization, even on the basis of single examinations of stools, the most common findings being the ova of Ascaris lumbricoides, Necator americanus and Trichuris trichiura, alone or in combination. No close correlation between the extent of hookworm infection and the degree of anemia was present in this series. The Kahn test disclosed only two doubtful and no positive reactions, but patient 9 had a hospital record of antisyphilitic therapy of one year's duration. Suspensions of the Negro patients' blood cells examined for the sickling trait did not reveal it. Icteric indexes were within normal limits. Sedimentation rates (Wintrobe) revealed no abnormalities when corrected for the anemic state.

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1. Yater, W. M.: Fundamentals of Internal Medicine, ed. 2, New York, D. Appleton-Century Company, Inc., 1944, p. 85. Gambill, E. E.; Hines, E. A., Jr.: Am. Heart J. **28**:763, 1944.

Analysis of the dietary histories secured from 23 patients revealed no nutritional deficiency common to all of the patients who were studied, but showed considerable individual variation as to qualitative and quantitative food intake. Major William F. Ashe, Medical Corps, Army of the United States, assistant director of the Nutrition Division, Office of the Surgeon General, United States Army, examined our dietary data and rendered the opinion freely quoted. We are convinced that the dietary histories of these patients are representative on the average of those of the general population, i. e., adequate to prevent frank clinical deficiency disease but not optimal.

Neither the patients included in this study nor those surveyed, nor those seen generally in hospitals, showed any clinical signs of vitamin deficiencies, such as blepharitis, xerophthalmia, keratomalacia, vascularization of the cornea, cheilosis, redness or other disfigurement of the tongue, follicular keratoses, erythema of the skin or neuritis.

MORBID ANATOMY OF TROPICAL ULCER AS OBSERVED IN GUATEMALA

Serial sections of the specimens were stained by the routine hematoxylin-eosin, Giemsa and Brown-Brenn methods for bacteria in all cases. Step sections were selected for iron staining; the Gallego method as modified by Lillie[2] was used

2. Paraffin sections, after being fixed in neutral 4 per cent aqueous solution of formaldehyde, are passed through the alcohols to water as usual.
Prepare solutions A and B:
A. Hematoxylin, 1 Gm.
95 per cent ethyl alcohol, 100 cc.
B. Solution of ferric chloride U.S.P., 4 cc.
Concentrated hydrochloric acid, 1 cc.
Distilled water, 95 cc.
Mix freshly prepared solutions A and B before use and stain sections six minutes.
Rinse in tap water.
Mordant in:
Distilled water, 200 cc.
Concentrated nitric acid, 1.5 cc.
Solution of formaldehyde U.S.P., 1.0 cc.
Solution of ferric chloride U.S.P., 1.5 cc.
} 30 seconds.
Rinse in tap water.
Stain in:
Carbol fuchsin, 1 per cent solution, 3.0 cc.
Acetic acid, 0.2 per cent aqueous solution, 50 cc.
Mix fresh.
} 5 minutes.
Rinse in distilled water.
Mordant as above—2 minutes.
Stain in trinitrophenol–aniline blue:
Aniline blue, 0.1 Gm.
Saturated aqueous solution of trinitrophenol (picric acid) 100 cc.
} 1 minute.
Rinse in 0.1 per cent aqueous solution of acetic acid.
Dehydrate and clear in acetone, acetone plus xylene, xylene (2 changes).
Mount in balsam.

for connective tissue and for differentiation of elastic tissue; the Masson trichrome stain was used for smooth muscle–connective tissue specific coloration.

For convenience of description the specimens in this series will be grouped as "early" if the duration of ulceration had been about two weeks to two months, "moderately advanced" if the duration had been from a few to several months, and "late" if the duration had been from one to several years.

Epidermis.—Epidermal hyperplasia was present in all specimens, being slight and regular in the earlier ones and progressing to fivefold and even tenfold increases of thickness in the late ones. At the margin of the ulcer in the early stages marked pseudoepitheliomatous hyperplasia was seen (fig. 1 *F*) but no cancerous proliferation. In the moderately advanced and late stages buds of reepithelization were noted at the borders, usually thin, and incomplete even in a single section. Occasionally a late specimen showed marked acanthosis at the edge of the ulcer. Hyperparakeratosis was always present at the border, grading to hyperkeratosis more distally, with considerable variation between cases as to degree, and apparently independent of the time element. Flattening of the epidermal surface was another constant feature in this series. Polymorphonuclear leukocyte infiltration of the epidermis, even to the formation of minute intraepidermal abscesses, was an inconstant finding, but as a rule was more pronounced in the early specimens. Decrease of pigment in the basal layer of the epidermis appeared to be a process continuous with the age of the lesion, which finally shows no recognizable pigment cells. (Compare with the clinical description in the foregoing section and with figure 1 *E*.)

Corium.—The papillae showed elongation, edema, broadening, and blunting of the apexes, thus contributing to the flattened contour of the epidermal surface. In the early stages the contents of the papillae consisted mainly of edema fluid, loosened connective tissue, dilated capillaries and hemorrhagic foci. Later there was fibroblastic proliferation increasing to complete fibrosis. There were scattered infiltrating polymorphonuclear leukocytes, with round cell and eosinophilic cell clusters, in the papillae in the early stages; in the later ones the papillae were devoid of leukocytes. Dilatation and tortuosity of capillary loops in the papillae were seen in all sections but were most pronounced in those from the early specimens.

In general, marked exudation of polymorphonuclear leukocytes was seen in the corium in the early stages; exudation predominantly of lymphocytes, monocytes and plasma cells in the moderately advanced ones, and progressively decreasing numbers of those cells in the later stages. The densest leukocyte accumulations were located near the ulcer borders, and graded distally. Foci of recent hemorrhage, siderotic patches, and phagocytes filled with ingested erythrocytes or iron pigment were seen in all of the series with considerable variation between levels of the same specimen. Basophilia was common, scattered at all levels of the corium and the subcutaneous fat and in the granulation tissue of the ulcer bed. Early there was leukocytic disruption of the elements of the pilosebaceous apparatus leading to absolute reduction in numbers in late stages. Foreign body giant cells marked the sites of some destroyed hair shafts, follicles and sebaceous glands. The sweat coils were dilated, had inspissated contents and showed periglandular round cell infiltration and edema. The late specimens showed dense fibrosis of the corium and the subcutaneous fatty layer with disappearance of sweat coils and hair follicles. The nerve bundles in the corium were anatomically normal in some sections; in others they revealed interstitial fibrosis, perineural round cell infiltration and in moderately advanced stages of the ulcer showed,

in addition, whorling of the nuclei in an almost neuromatous manner. In 2 such cases the nerve segments showed greatly distorted, deeply staining, swollen nuclei; in another, specific staining revealed the myelin disrupted into droplets. The conclusion appears warranted that these nerve changes are the cause of the relative anesthesia noted clinically in the ulcer areas (see section entitled "Clinical Aspects").

Subcutaneous Fat.—Serous atrophy, exudation of moderate numbers of polymorphonuclear leukocytes and round cells and, in occasional minute foci, necrosis with foreign body giant cell reactions were seen in the subcutaneous fat.

Area of Ulcer.—The ulcerous area underwent minor changes with duration of the lesion. In all stages it consisted of a highly vascularized granulation tissue with the following variations: On the surface was a fibrinopurulent membrane varying from a hardly discernible layer to one the thickness of the epidermis, overlying an irregular zone of hyaline necrosis, sometimes very thin and at others extending for about one quarter of the thickness of the granulation tissue bed. As a rule, the capillaries of the granulation tissue extended to the very surface and early were supported by a very loose fibroblastic matrix; in the later stages fibrosis separated the exuberant vessels into clusters. The inflammatory exudate tended to be predominantly of polymorphonuclear leukocytes in the early specimens and more of the lymphocytic, monocytic and plasma cell type in the later ones. Eosinophil and basophil leukocytes were numerous in early specimens.

Vascular Involvement.—The distinguishing feature of all these specimens of tropical ulcer lay in the vascular changes encountered. Arteries, to a lesser extent veins, of all calibers were thickened, their lumens narrowed, sometimes obliterated, occasionally recanalized. The capillaries showed similar changes. Naturally, the vessels of larger caliber were not represented in a few biopsy specimens simply because the block failed to cut across vascular channels of such caliber.

Pathologically, the specimens were different in the three main divisions of the blood supply, namely the arteries, the veins and the capillary bed. The larger, medium-sized and small arteries seen (fig. 2 *A* and *B*) showed marked thickening of the media by muscular hyperplasia, with moderate to marked stenosis of the lumen. Such vessels frequently had an intact, well defined internal elastic membrane. In other thickened vessels the internal elastic membrane was split and frayed and had either a band of smooth muscle or a mixture of smooth muscle and collagenous connective tissue central to it, i. e., subintimal fibrosis or simple subintimal hyperplasia. In a few instances the internal elastic membrane was found split and frayed in the middle of a greatly hypertrophied muscular media (fig. 2 *C*). There was a greatly thickened adventitia as well in many of the smaller arteries. In some cases all of these changes were represented in different vessels at various levels of the same block. In other cases only the commonest of the arterial lesions was seen, namely medial muscular hyperplasia. Serial sections demonstrated the focal nature of the lesion in its extreme form, but rarely did any level demonstrate a restitution of the normal picture.

The larger and medium-sized veins showed inconstantly but frequently either great muscular hyperplasia, with narrowing of the lumen, or a mixture of hyperplasia and interstitial fibrosis. Occasionally a vessel was observed in which the collapse of a wall was outlined by strands of smooth muscle and elastic fibers surrounding a loose collagenous meshwork showing recanalization by capillaries. Or a portion of a venous wall was seen in which occurred a condensation of elastic fibers, an absence of smooth muscle and an admixture of fibroblasts and

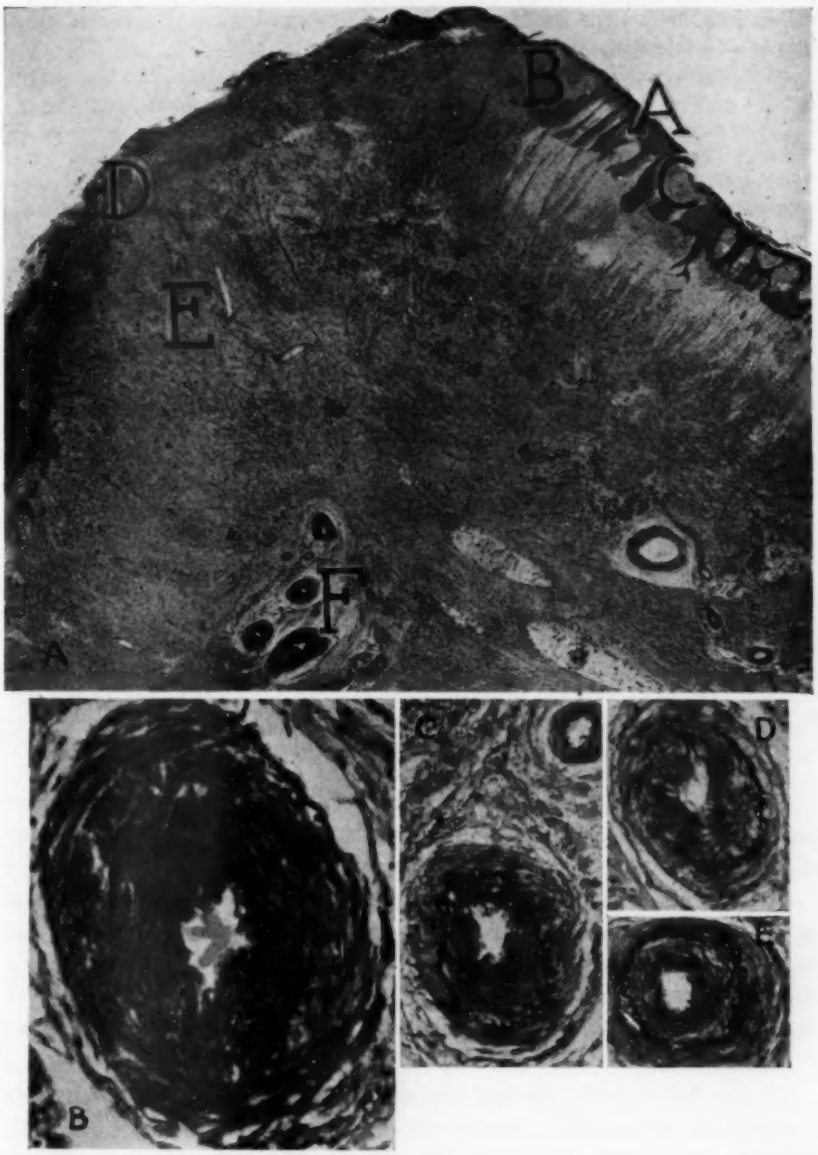

Fig. 2.—*A*, topographic view of a biopsy specimen of tropical ulcer (patient 5) showing the great thickening of the entire skin, the flattened contour of the epidermal surface (*A*), the epidermal hyperplasia (*B*), the broadening and lengthening of the papillae (*C*), the fibrinopurulent membrane over the ulcer surface (*D*), the granulation tissue bed of the ulcer (*E*) and the greatly thickened vessels with narrowed lumens at the level of the subcutaneous adipose tissue (*F*); × 16. (Army Medical Museum negative, no. 89820.) *B*, vessel shown at ***F*** in part A; × 230. Note the great medial hyperplasia and narrowed lumen. (American Medical Museum negative, no. 89815.) *C*, vessels (*F* in part *A*) stained by the Gallego method as modified by Lillie; × 235. The frayed internal elastic laminas now occupy midpositions in the media. (Army Medical Museum negative, no. 89815.)

collagenous fibers. The smaller veins frequently showed not only muscular hyperplasia but adventitial fibrosis.

Not infrequently patchy hyalinization of the wall was seen affecting chiefly, but not exclusively, the smaller arteries, even forming a concentric band (fig. 3). Hyalinization was seen most commonly and fairly uniformly in this series in the capillary bed, where endothelial hyperplasia and hypertrophy were common, resulting in vessels of four and even six endothelial layers. Between such hyperplastic endothelial cells there was often a layer of hyalin (fig. 3). Hyperplasia of endothelium obliterated the lumens of many capillaries.

Vessels of arteriolar caliber showed perhaps the most marked stenosis of any portion of the vascular tree examined, along with muscular hyperplasia, chiefly, and sometimes adventitial fibrosis as well. No tissue preserved in the wet state was available for fat determinations, so it is impossible to state with certainty that the fine vacuolation seen in some such vessel walls represented lipoid infiltration. In one medium-sized artery large lipoidal cells of signet ring form were plentifully distributed through the media.

The following changes were seen once or only a few times in the sections examined: Medium-sized and small arteries showed partial fibrous replacement of the hypertrophied medial coat with and without foci of a gelatinous type of necrosis. Some small veins showed fairly thick bands of subintimal fibrosis narrowing and puckering the lumen. Thrombi, occlusive and nonocclusive, usually fresh, but definitely attached, were seen most frequently in the dilated capillaries of the granulation tissue, but were also seen isolated in one small vein. Lastly, in a few instances special stains demonstrated that clusters of small vessels deep in the corium were in reality a recanalization process in a totally destroyed vessel. A single instance of complete hyalinization of a vessel wall was seen in our oldest specimen, in which a cluster of endothelial cells surrounding a minute lumen was all that remained of the vessel.

The question naturally arises whether these vascular changes are secondary to the inflammatory picture described. The evidence in favor of a primary vascular disease may be summarized as follows:

1. Vascular lesions, including those of greatest severity, were found in all cases of tropical ulcer in an early stage, including those of ten days' to two weeks' duration (fig. 3). In fact, those of years' duration showed great vascular obliteration, apparent only in remnants of smooth muscle and elastic fibers embedded in a much scarred layer of corium or of subcutaneous adipose tissue.

2. In the sections studied the most pronounced vascular changes were found away from the zones of greatest inflammatory exudate, such as the deeper layers of the corium, the subcutaneous adipose tissue and the distal edge of the biopsy block. Very frequently the vessels showing these changes lay in areas entirely devoid of inflammatory exudate, and only rarely were they seen embedded in dense leukocytic pools (fig. 3).

3. One of the two usual vascular diseases secondary to inflammation, whether acute or chronic, suppurative or granulomatous, is endarteritis or endophlebitis, frequently with occlusion. The vascular disease

described in the foregoing paragraphs is entirely different from those entities. The other vascular disease seen secondary to acute or chronic inflammation is vasculitis. The only vessels of this series showing that condition were the much enlarged capillaries of the granulation tissue bed, where acute vasculitis and the deposition of thick, irregular hyaline collars were the rule (fig. 3). These changes are not interpreted as of significance equal to that of the changes described in the foregoing paragraphs, being seen in a variety of inflammatory lesions and in ulcers of varied cause, such as varicose ulcers and those following diabetic arteriosclerosis and trauma.

We have not encountered a similar vascular pattern in any other lesion known to us. That the vascular damage of arteries, veins, arterioles and capillaries should be considered together as a unit seems to us an inescapable conclusion from their association in all cases. It is conceivable that more than one factor operates in the genesis of these vascular abnormalities, a subject which we explored only lightly [see later comment].

By way of making control observations specimens of the skin of the anterior tibial and lateral surfaces of the lower third of the leg were removed from patients coming to routine autopsy in Guatemala who were of the same racial type as most of our patients with ulcer. Males and females, ranging in age from 20 to 50 years, totaling 13, were studied for vascular abnormalities by serial section of specimens of skin of the leg. No vascular changes were found comparable to those of the series with tropical ulcer. (Figure 3 shows typical vessels of the control group.) However, at an autopsy a specimen was secured, consisting of a wide, deeply pigmented zone of the lower extremity, in which there were a few small opaque white plaquelike scars. It is conceivable that this represented a healed tropical ulcer of Guatemala. At any rate, sections of this specimen showed vascular changes comparable in kind but not in degree to those seen in specimens from patient with the ulcer.

One of the other causes of ulceration common in Guatemala is cutaneous leishmaniasis, which does not show any predilection for the lower extremities. Four biopsy specimens from patients with this disease were secured, all representing lesions of many months' duration. They showed no vascular abnormalities at all comparable to those of the tropical ulcer series. Furthermore, they showed a pronounced granulomatous inflammation differing again from that seen in the tropical ulcer series. Leishmania forms were identified after prolonged search in one section from each of these serially sectioned specimens.

Another biopsy specimen was secured in Guatemala from a varicose ulcer of a lower extremity of a man who had had varicosities for twelve years and the ulcer for about one year. The Trendelenburg test showed reflux filling of the distended veins from above downward. The varicosi-

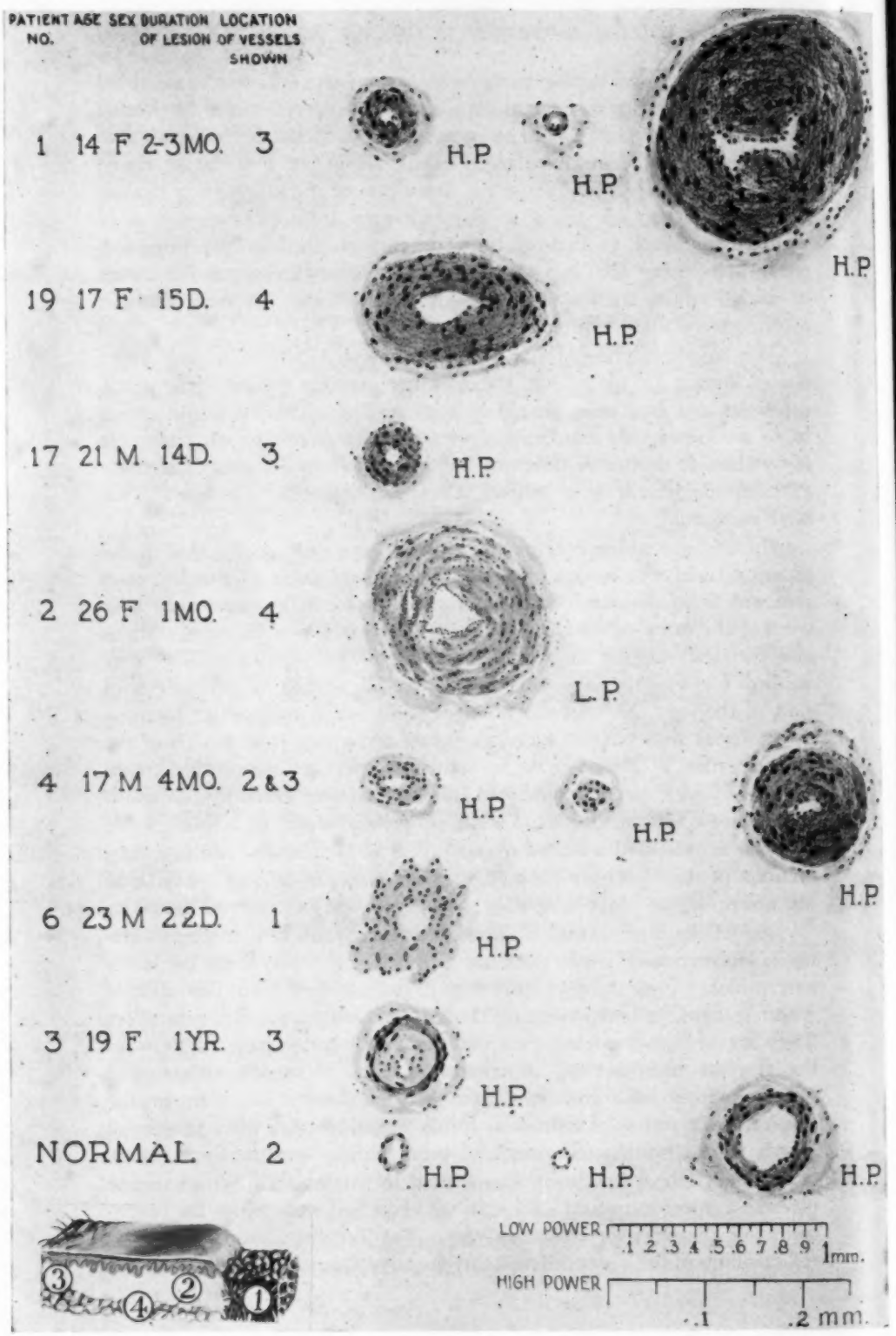

Figure 3

(See legend on opposite page)

ties were plainly visible and markedly severe. There were gross differences between this specimen of ulcer and those of our tropical ulcer series. The microscopic sections of the varicose ulcer showed none of the vascular changes described by us as a distinguishing feature of tropical ulcer.

From the files of the pathology laboratory of the National Institute of Health, Bethesda, Md., were selected 5 cases [3] of ulceration of the lower leg originating in the continental United States in patients ranging from 13 to 59 years of age, none of which showed comparable vascular lesions. In a sixth case,[4] ulceration of the lower leg from the same source occurred in a steamship wireless operator who had spent ten months in the South Pacific Area. The vessels seen in one of the biopsies were only of small artery and arteriolar caliber but were comparable to those seen in our tropical ulcer series in their thickenings and luminal stenoses.

Inasmuch as other investigators (see the following section) have reported fusospirillosis of the type observed in Vincent's infection as the causative agent of tropical ulcer, one of us (A. G.) examined a series of 46 biopsy specimens secured by Dr. E. Hampp,[5] passed assis-

3. S-21140, S-21057, S-20478, S-19991, S-19812.

4. S-19642, and S-19396. All of these cases were studied with the permission of Dr. R. D. Lillie.

5. Unpublished data.

EXPLANATION OF FIGURE 3

Camera lucida drawings of affected blood vessels. The patients are those of the same numbers listed in tables 1 and 2. The location of the vessels illustrated is given in numerical zones, which are diagramatically represented in the figure at the lower left hand corner. *H. P.* signifies high power (× 440) and *L. P.* low power. (× 100).

Patient 1. The medium-sized and large vessels (arteries) show muscular hyperplasia of the media and marked stenosis of the lumen. The smallest vessel is a capillary showing endothelial hyperplasia and hyaline thickening of the wall.

Patients 17 and 19. A small artery and an arteriole are illustrated, respectively, both showing muscular hyperplasia of the media with encroachment on the lumen.

Patient 2. This is a medium-sized vein showing muscular hyperplasia of the wall and interstitial fibrosis of the media, resulting in an irregular, narrowed lumen.

Patient 4. The two smaller vessels are capillaries showing extreme hyaline thickening of the walls and marked endothelial proliferation. The largest vessel is a small artery with lesions like those already described.

Patient 6. This is a capillary in the granulation tissue with a thick, irregular hyaline collar around it, permeated by polymorphonuclear leukocytes.

Patient 3. The stenotic lumen of this vessel is surrounded by a wide band of hyaline necrosis within the muscle coat.

"Normal" represents a group of three vessels as seen in one of the control sections of skin removed at autopsy from the lower third of the leg.

tant surgeon, United States Public Health Service, in known cases of Vincent's gingivitis. None of these sections showed any vascular abnormalities at all comparable to those of our tropical ulcer series.

Bony Involvement.—Another feature of tropical ulcer occurring in this region is the eventual involvement of bone that takes place in the chronic cases, i. e., those of many months' to years' duration. Two patients with involvement of bone are included in this series, patients 20 and 27. In the former a roentgenogram taken prior to operative exploration showed marked cortical opacity and thickening which encroached on the medullary canal, periosteal thickening and fine opaque radial lines, which were interpreted as calcifications. Microscopic sections of the bone showed greatly thickened lamellas, without evidence of active osteoclasia or osteoplasia. The marrow elements were reduced to a loose collagenous meshwork, in which very small numbers of round cells were identified. There was no evidence of osteomyelitis.

Negative Findings.—There are a number of significant negative findings that require emphasis; no granulomas other than the small numbers of foreign body giant cells described in a foregoing paragraph (page 619) were encountered; no fungous forms were found; no inclusion bodies were seen; no parasites were found. The female chigoe (Tunga penetrans) is a common parasite in Guatemala infecting as a rule the toes, occasionally the hands, in a characteristic paranychial distribution. It so happened that none of the patients in this series was infected by chigoes, and none was seen grossly or microscopically in the specimens of tropical ulcer. The vascular changes described and illustrated served to differentiate this from the other ulcers of the area studied.

MICRO-ORGANISMAL FLORA OF GUATEMALAN TROPICAL ULCER

According to the general plan of procedure already outlined, cultures were made on blood agar aerobically and anaerobically (Wright's method). Increased carbon dioxide tension was used for blood agar slants in several cases but showed no advantages and its use as a routine procedure was later discontinued. Robertson's cooked meat medium (with 0.5 per cent tryptose added; sealed with a petrolatum plug), brain broth medium (Bacto), semisolid thioglycollate stab preparations (Brewer's) and potassium tellurite–blood agar plates were also used.

The results are summarized in table 1. Different varieties of staphylococci and streptococci were identified, with no constancy as to species, along with a number of coccal and bacillary forms known to be nonpathogenic. No significance could be attached to differences in flora in those several instances in which excised corium and ulcer surface were cultured separately. The varying character of the bacterial flora was brought out clearly in a series of 26 cases in which smear preparations were made directly from the ulcer, in 20 of which special mediums were used for the isolation of organisms of the Corynebacteria group (see table 2 for a report of the latter). In contrast to the reports of many investigators in other parts of the world, notably Clements,[6] who found Vincent's

6. Clements, W.: M. J. Australia **2**:615, 1936.

fusiform bacillus–spirochetal complex regularly in tropical ulcer, we found it not at all as a complete picture. Occasionally spirilla and occasional bacillary forms were found admixed among coccal and fine bacillary forms. The varicose ulcer described on page 623 and a bacterial flora similar to that of the tropical ulcer series, consisting, namely, of Staphylococcus aureus and Staphylococcus epidermidis.

Cultures for fungi were made from triturated portions of the biopsy specimens. In a few cases cultures were made by mincing a part of the specimen with sterile scissors, and in the remainder cultures were made also from the surface of the ulcer.

The inoculum was streaked on the surface of Sabouraud's agar slants. Deep shake cultures on dextrose–veal infusion–agar also were made in a few of the cases. Cultures were incubated two months before being discarded.

In most of the cases a yellow staphylococcus was isolated. There were no consistent mycologic findings, and it was concluded that the few miscellaneous molds cultured were accidental contaminants. The results of the attempts to isolate fungi are given in table 2.

TABLE 2.—*Results of Attempts to Isolate Fungi from Areas of Tropical Ulcer in Twenty-Four Patients*

Patient	Fungi	Patient	Fungi	Patient	Fungi
1	0	9	0	19	Candida tropicalis
2	0	10	0	21	0
3	0	11	0	22	0
4	0	14	0	23	Cladosporium SP.
5	0	15	Monilia SP.	24	0
6	0	16	0	25	Neurospora sitophila
7	0	17	Streptomyces	27	Sterile gray mold
8	0	18	0	29	0 (Varicose ulcer)

The tissue sections were examined for micro-organisms by the Brown-Brenn and the Giemsa method, with morphologic findings consistent with those listed in table 1. Most of the organisms seen in tissue sections were on the surface in sparse numbers, occasionally in minute clumps. Small numbers of exclusively gram-positive cocci could be seen deep in the granulation tissue bed of the ulcer area, and occasionally phagocytosis. Considerable difficulty was experienced in differentiating some basophils and cells with granular debris in their cytoplasm from phagocytes with ingested bacteria. Usually tissue basophils with small uniform granules had a purplish hue with both the Giemsa and the Brown-Brenn method, while the gram-positive bacteria were definitely blue with both methods. Ten sections from 10 patients were stained by the Warthin-Starry method for spirochetes in an attempt to find organisms of Vincent's complex. None were seen.

Even though the organisms isolated from these patients were of the usual skin-contaminant group it was deemed essential to test their pathogenicity. Inoculations of pure cultures of isolated staphylococci and streptococci and of both organisms together were made intracutaneously in guinea pigs and rabbits. All failed to produce any lesion other than a temporary inflammatory zone without ulceration or any entity at all comparable to the tropical ulcer studied in man. Furthermore, in all the animals the lesions healed in several days to a few weeks at the most, thus differing further, in lack of chronicity, from the human lesions; 35 guinea pigs, 16 rabbits and 1 monkey were used in these experiments.

Proceeding on the possibility that a noncultivatable agent, such as a filtrable virus, might be present, 1 monkey (Macacus rhesus), 4 rabbits and 4 guinea pigs were inoculated with fresh suspensions of 4 pooled triturated biopsy specimens of tropical ulcer without producing any lesions whatsoever in thirty days of observation.

It was decided to make a special search for organisms of the Corynebacteria group, using a series of patients with tropical ulcer in one hospital, not previously studied. Of 20 patients surveyed, 1 was found harboring diphtheria organisms pathogenic for the guinea pig, confirmed by autopsy observation of typical myocardial lesions and extreme adrenal hemorrhage. This patient's ulcer differed not at all clinically or grossly from those previously studied. Nor did this patient have any clinical signs of diphtheria intoxication. One hundred thousand units of diphtheria antitoxin was administered intramuscularly in a single dose to this patient after the character of the isolated micro-organisms had been established. There was no clinical improvement in the ulcer thereafter over a two week period of observation. Time did not permit us to reassay the lesion for diphtheria organisms after administration of the specific antitoxin.

It appeared entirely consistent with the observed facts to conclude that the micro-organisms isolated were harbored by the lesions but were not etiologically related. The lack of constant bacterial types or even of common combinations of organisms in all lesions supported this conclusion. The demonstrated lack of pathogenicity of the organisms inoculated in experimental animals was further support for this thesis.

Proteus agglutinations were undertaken with stock cultures of O X 19, O X 2, O X L and O X K, which revealed no significant titers for any of the serums, although cases of months' and even years' duration were included in this series. This does not necessarily exclude an organism of the Rickettsia group from etiologic consideration, since species of the Q fever type, for example, do not react with the Proteus antigens.

COMMENT

Of the experimental evidence presented no one type stands out as clearly as the vascular, seen in all cases on microscopic examination of the tissue sections. It is the factor common to all. Furthermore, the vascular damage is sufficiently severe to warrant the conclusion that there is serious interference with the blood supply of the region. Such a circulatory deficiency is known to result in ulceration whether it be on the basis of arteriosclerosis or that of varicose veins.

We were unsuccessful in our search for factors productive of the vascular changes. Hypertension could be dismissed as an etiologic agent by reason of our measurements which, even though they were single readings, covered a much larger group than that represented in this series. Because our patients so frequently gave a history of "fly bites," we attempted to produce experimental insect sting trauma. Guinea pigs and rabbits were subjected to the bites of the tick Boophilus emulatus; others were observed while laboratory bred Triatoma fed fully on their shaved skin; another was stung by a honey bee. In all not even an appreciable wheal was produced. Before surrendering this bit of speculation, we wish to point out that insect bites sustained

by one of us (A. G.) in the field were frequently much more severe on the lower legs than anywhere else on the body. As a general observation, the population of the lowlands as seen in market places, at work and in dispensaries tended to show more severe reactions to insect bites on the lower extremities than elsewhere on the body. Whether the intensity of these reactions is correlated with some vascular peculiarity of the region remains to be proved. Earle [7] quoted Rauber and Kopsch to the effect that the integument of the lower third of the leg is supplied by terminal vessels of adjoining arteries. Whatever may be the underlying anatomic basis, if any exists, the fact remains that the bodily region so commonly affected by tropical ulcer is vulnerable to the consequences of vascular insufficiencies whether these are produced by low temperatures, occulsive arteriosclerosis or failure of adequate venous return.

March and Wilson [8] summed up their experience with tropical ulcer as observed in Iran with the "alliterative mnemonic" as to etiology: "Filth, food, friction, and fusospirillosis." We were not impressed with lack of cleanliness in our patients. In fact, many showed reasonable maintenance of hygienic conditions. "Food," meaning various nutritional deficiencies, has been discussed at length by many authors, among whom Earle,[7] studying tropical ulcer in Trinidad, postulates deficiencies of dietary animal protein, animal fat, calcium, phosporus and vitamins A, B_1, B_2, C and D as contributory factors in the genesis of the lesion. Clements [6] incriminated as one of the causal agents another dietary complex as seen in New Guinea: partial or complete deficiency of the B_2 complex, abundance of carbohydrates and only small amounts of proteins and fats. Charters [9] expressed the belief that vitamin A deficiency is a precursor of the "specific infection" of tropical ulcer. Whatever validity those dietary observations have they fail to agree as to specificity. In addition, our dietary findings are not in agreement with them. Our patients ate what can be compared biochemically to a diet encountered among the poor of the Southern states. It appears reasonable to inquire why, if dietary deficiency is the cause of tropical ulcer, it is not seen in that area. Furthermore, if vitamin A deficiency, for example, is a significant contributing factor, why is tropical ulcer not seen in many other parts of the world where that deficiency is certainly not unknown? If all that is needed is superimposed bacterial infection, the micro-organismal flora seen by us and that seen by others as fusospirillosis is ubiquitous.

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7. Earle, K. V.: Tr. Roy. Soc. Trop. Med. & Hyg. **35**:241, 1942.

8. March, F., and Wilson, H. A.: Tr. Roy. Soc. Trop. Med. & Hyg. **38**: 259, 1945.

9. Charters, A. D.: Tr. Roy. Soc. Trop. Med. & Hyg. **37**:205, 1943.

Our method of clinical and laboratory examination of the patient, survey of the micro-organisms and microscopic study of the tissues secured by biopsy has yielded significant information as to the character of tropical ulcer as observed in one geographic area. We have no experience with it as seen in other parts of the world. Consequently we cannot generalize on our findings or criticize the findings of those working in other countries.

The therapy of tropical ulcer has been directed toward bacterial stasis or antisepsis, without great success. It would appear from our observations that methods aimed at restoring the adequacy of the blood supply of the leg would prove more fruitful therapeutically.

SUMMARY

The tropical ulcer of Guatemala orginates in the lowlands. In this study designed to secure data on its genesis, clinical methods and clinical laboratory methods revealed nothing of fundamental importance. Bacteriologic and mycologic technics demonstrated a micro-organismal flora of nonuniform type, regarded as adventitious. Fresh ulcer tissue and pure growths of the isolated bacteria when inoculated in rabbits, guinea pigs and a monkey produced no lesions at all comparable to the tropical ulcer of the area. The significant lesion in these cases of tropical ulcer was found on microscopic examination of biopsy specimens and consisted of widespread damage of the venous and arterial supply. While the vascular abnormalities were of variable anatomic type they had one characteristic in common: stenosis of the lumen. An argument is presented for regarding these vascular abnormalities as the primary cause of this ulceration.

ETHYLENE GLYCOL POISONING

With Suggestions for Its Treatment as Oxalate Poisoning

COMMANDER GEORGE MILLES (MC), U.S.N.R.

THE GLYCOLS are stock commercial solvents, and ethylene glycol is freely accessible to the general population in the form of an antifreeze solution. The toxicity of this group of compounds was forced on the public consciousness by the fatalities which occurred incident to the use of a solution of sulfanilamide in diethylene glycol. This compound, in common with others of the group having an ether linkage, causes hydropic degeneration of the renal tubular epithelium.[1]

The toxicity of ethylene glycol has not received the publicity that it merits. Indeed, the containers in which this glycol is marketed bear no indication of the fact that it is poisonous. In 1927 Page [2] published a pharmacologic study of this compound which indicated that it was relatively nontoxic. He drank 15 cc. without ill effect. He reported that dogs tolerated a 30 per cent solution in a dose of 9 cc. per kilogram of body weight without apparent effect and that the concentrated product in a dose of 5 cc. per kilogram of body weight caused only emesis. He stated that because of the structure of ethylene glycol it would not seem unlikely that the compound would be converted to sugar in the animal body.

In 1930, in a letter [3] reporting the death of 2 men following their ingestion of ethylene glycol (Prestone) it was stated: "Death seemed to have been caused by respiratory failure and convulsions." The victims were first seen twelve hours after they had drunk diluted antifreeze solution. They were stuporous; their respirations were rapid; the skin was cold and somewhat cyanotic. Vomiting was persistent, and prostration was extreme. The anatomic findings were not reported.

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This article has been released for publication by the Division of Publications of the Bureau of Medicine and Surgery of the United States Navy. The opinions and views set forth are those of the writer and are not to be considered as reflecting the policies of the Navy Department.

1. Kesten, H. D.; Mulinos, M. G., and Pomerantz, L.: J. A. M. A. **109**: 1509, 1937.

2. Page, I. H.: J. Pharmacol. & Exper. Therap. **30**:313, 1927.

3. Possible Death from Drinking Ethylene Glycol ("Prestone"), Queries and Minor Notes, J. A. M. A. **94**:1940, 1930.

Brekke [4] reported 2 cases of ethylene glycol poisoning, with recovery following decapsulation of the kidneys. The initial symptoms were similar to those of alcoholic intoxication. They were followed by paralysis of some cerebral nerves, hemorrhagic nephritis, acute uremia and marked hyperemia and swelling of the kidneys. Wiley and associates [5] found that dogs and rabbits oxidized glyoxal, glycolic acid, ethylene glycol and ethylene glycol monacetate to oxalic acid.

Laug and associates [6] stated that ethylene glycol caused congestion and hemorrhage of the lungs, albuminuria and hydropic degeneration of the tubular epithelium of the kidneys.

Sollmann [7] described ethylene glycol as being twice as toxic as propylene glycol and half as toxic as diethylene glycol. He stated that when ethylene glycol is oxidized to oxalic acid, but a small fraction of that consumed appears to be involved and that oxidation cannot account for its toxicity.

Lehmann and Flury [8] stated that many authors have ascribed the effect of ethylene glycol to the formation of oxalic acid and that 3 per cent of the ingested ethylene glycol can be detected in the urine in the form of oxalic acid. They concluded that because it is oxidized to oxalic acid, ethylene glycol is more toxic than propylene glycol, since the latter is oxidized to pyruvic acid or lactic acid, and is eliminated in combination with glycuronic acid. They described the effect of large doses of ethylene glycol as exciting and later depressing the central nervous system, and the lethal dose as ranging from 5 to 9 cc. per kilogram of body weight of mice, rats and rabbits, the commercial being more toxic than an especially highly purified product.

Boemke [9] reported an instance in which a bucketful of antifreeze solution was substituted for the coffee water in a German military unit. It was noted that the coffee had a sweetish, somewhat bitter taste. During the course of the morning various members of the unit complained of headache and became intoxicated, for which they thought the coffee responsible. Two of the men who drank especially large amounts of the coffee were admitted to the hospital, severely intoxicated, dizzy and dyspneic. Their alae nasi moved actively, their facial expression

4. Brekke, A.: Norsk mag. f. lægevidensk. **91**:381, 1930.

5. Wiley, F. H.; Hueper, W. C., and von Oettingen, W. F.: J. Indust. Hyg. & Toxicol. **18**:123, 1936.

6. Laug, E. P.; Calvery, H. O.; Morris, H. J., and Woodward, G.: J. Indust. Hyg. & Toxicol. **21**:173, 1939.

7. Sollmann, T.: A Manual of Pharmacology, ed. 6, Philadelphia, W. B. Saunders Company, 1942, p. 941.

8. Lehmann, K. B., and Flury, F.: Toxicology and Hygiene of Industrial Solvents, Baltimore, Williams & Wilkins Company, 1943, p. 254.

9. Boemke, F.: Virchows Arch. f. path. Anat. **310**:106, 1943.

was one of fright and their reflexes were depressed. One, who was 30 years old, rapidly lapsed into unconsciousness, became anuric and died on the day following his admission. At autopsy the findings were not distinctive. Microscopically, the kidneys alone were noteworthy. The tubules were dilated, and the tubular epithelium contained finely divided fat droplets. The lumens contained hyaline material, erythrocytes and crystals of various types, stained faintly blue with hematoxylin. The second patient, who was 24 years old, improved somewhat under symptomatic treatment. He passed small amounts of urine containing albumin and red blood cells but no oxalate crystals. He became anuric, lapsed into unconsciousness and died ten days after ingesting the contaminated coffee. At autopsy the findings were hemorrhagic bronchitis and bronchopneumonia, fibrinous pleurisy of the right lower pulmonary lobe, dilatation of the right side of the heart and edema of the brain. The kidneys were swollen and pale. The microscopic changes in the kidneys were similar to those described in the first case. Since these men were truck drivers, carbon monoxide poisoning was suspected at the time of their admission, and spectroscopic examination of the blood disclosed the presence of carbon monoxide hemoglobin. However, the coffee was shown to contain 10 per cent ethylene glycol, and in view of the presence of oxalate crystals in the kidneys, this was recognized as the cause of death. The author referred to two other deaths which occurred one day after the ingestion of 750 cc. of ethylene glycol in which the anatomic observations were the same as in the cases just described. He ascribed deaths from the ingestion of ethylene glycol to the fact that this compound is oxidized to oxalic acid, which is deposited in the kidneys, causing anuria and uremia.

My interest in the toxicity of ethylene glycol was aroused by the following case:

REPORT OF A CASE

A 30 year old white man was admitted to the hospital with a history of having drunk antifreeze solution about four hours previously. During the interval prior to admission he became euphoric and vomited. On admission his voice was husky, his speech was thick and he was confused. The conjunctivas and the pharynx were injected, and the skin was cyanotic. The blood pressure was 128 systolic and 90 diastolic; the pulse was full and regular and the rate 90; the pupils were sluggish. About seven hours after he ingested the radiator fluid, his respirations were labored, and the rate was 36 per minute; the pulse had increased to 120, and he vomited foamy yellow fluid. Evidence of pulmonary edema became increasingly pronounced, and the patient lapsed into coma ten hours after drinking the antifreeze solution. At this time, the blood pressure was 172 systolic and 100 diastolic, respirations were sighing and rapid, and the fingers and the hands had become more deeply cyanotic, though the heart tones were good. Frothy, blood-stained fluid was aspirated from the air passages. Twenty-one hours after the ingestion of the radiator fluid the axillary temperature had risen to 101.4 F. The patient had voided 1,650 cc. of urine since his admission. It contained 20 mg.

of albumin and numerous oxalate and urate crystals. He died about twenty-two hours after ingesting the fluid.

Traces of ethylene glycol were demonstrated in the stomach contents aspirated about ten hours after its ingestion. A sample of the radiator fluid submitted for analysis was emerald green and was found to contain about 50 per cent ethylene glycol. No other common poison could be demonstrated. Inquiry indicated that the man had drunk not more than 500 cc. of the fluid.

Treatment was symptomatic and included intravenous injection of sodium *r*-lactate one-sixth molar N. N. R., to combat the apparent acidosis, and gastric lavage.

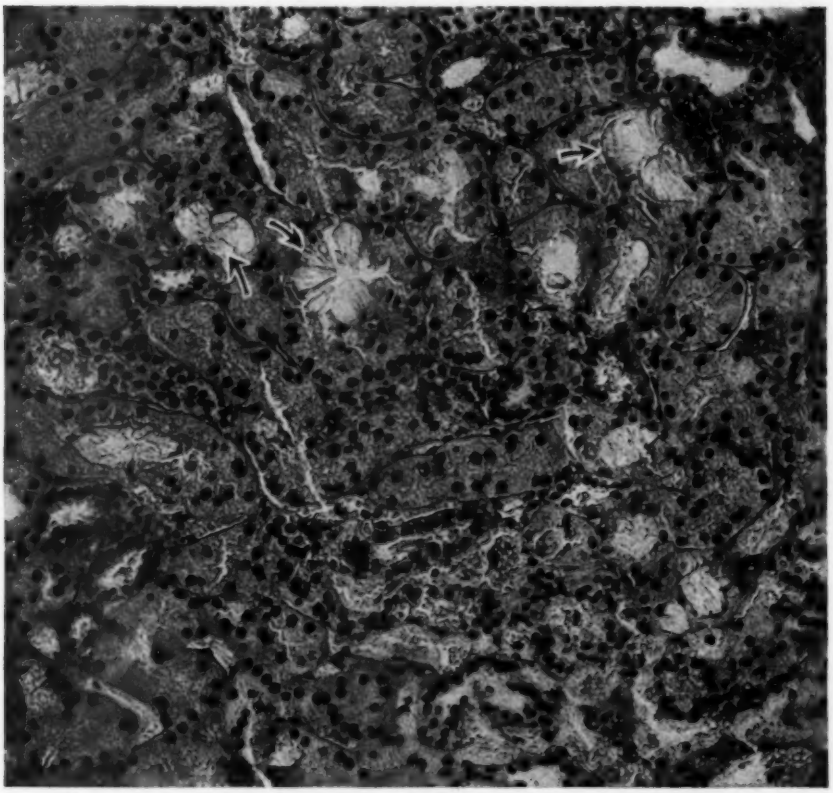

Fig. 1.—Oxalate crystals in the renal tubules of a 30 year old man dead from ingestion of ethylene glycol.

At autopsy, eight hours after death, the right pupil was slightly larger than the left, the conjunctivas were injected and edematous, and the skin and the mucous membranes of the head and the neck were cyanotic. The lividity of dependent parts was unusually pronounced. The heart weighed 410 Gm. and was distended with postmortem clots. The distal end of the trachea and both bronchial trees were filled with blood-stained serous fluid, and their mucosa was red. The lungs were boggy; their dark red cut surfaces pitted on pressure and exuded an abundance of blood-stained serous fluid. The lining surface of the

esophagus and stomach was homogeneously pale, opaque and grayish tan. There were a few mucosal hemorrhages in the stomach. Several blanched peanuts found in the esophagus and the stomach were stained the same shade of green as the fluid which was said to have been ingested. The stomach and small and large intestines were distended with light, muddy, greenish brown, thick fluid. The liver was light reddish brown mottled with yellow and was normal in size and consistency. The kidneys together weighed 350 Gm. They appeared grossly normal except for their rather pale color. The vessels of the pia-arachnoid were markedly congested. There was pronounced lipping of several thoracic vertebrae. The autopsy findings were otherwise not remarkable. Postmortem determinations of blood sugar and nonprotein nitrogen showed 34 mg. and 74 mg. per hundred cubic centimeters respectively.

On microscopic examination of the kidney, the glomerular tufts were seen to be moderately distended and their capillaries filled with red cells. Their interstitial tissue had a coarsely fibrillar, hyaline appearance; its nuclei were deeply stained and angular. Bowman's capsule was somewhat increased in thickness and hyalinized. The epithelium of the convoluted tubules was swollen except at the many points where the tubules were distended with colorless rosette and linear crystals. Similar crystals were present in the collecting tubules (fig. 1). The liver cells were swollen and honeycombed with fine fat vacuoles, which became confluent in the cells about the central veins. Their nuclei were variable. The Kupffer cells contained an abundance of brown granular pigment. The pulmonary alveoli were filled with serous material, and in focal areas they and their neighboring bronchioles were filled with an exudate of polymorphonuclear leukocytes. Congestion and, to a lesser extent, edema characterized the pathologic process in the other organs. Death was due to edema of the lungs, which may have been a condition secondary to an aggravating factor in or the cause of the marked myocardial dilatation and evident acute myocardial failure.

EXPERIMENTAL STUDY

The fluid which this man was said to have drunk was administered to several cats by stomach tube.

Cat 1.—A male cat weighing 3.25 Kg. was given a dose of 1 cc. per kilogram. Its condition as observed at intervals after administration of the fluid was as follows:

20 min.	Drowsy
30 min.	Less drowsy but apathetic
36 min.	Responsive to the smell of food (sat up)
46 min.	Apathetic, hiccupped
3 hr.	Dozing, apathetic
23 hr.	Apathetic, dozing, responsive to pain and noise, slightly unsteady of gait
27 hr.	Alert, tense, with good muscle coordination, somewhat apathetic
48 hr.	Normal

Cat 2.—A female cat weighing 2.4 Kg. was given a dose of 3 cc. per kilogram. Its condition as noted at intervals after administration of the fluid was as follows:

3 hr.	Slightly depressed
22	Practically normal
29	Irritable
46	Irritable, tender over abdomen and flanks
94	Slightly depressed, with no tenderness
118	Apparently normal
142	Comatose
144	Seized with clonic and tonic convulsions
146	Dead, with colorless froth at mouth

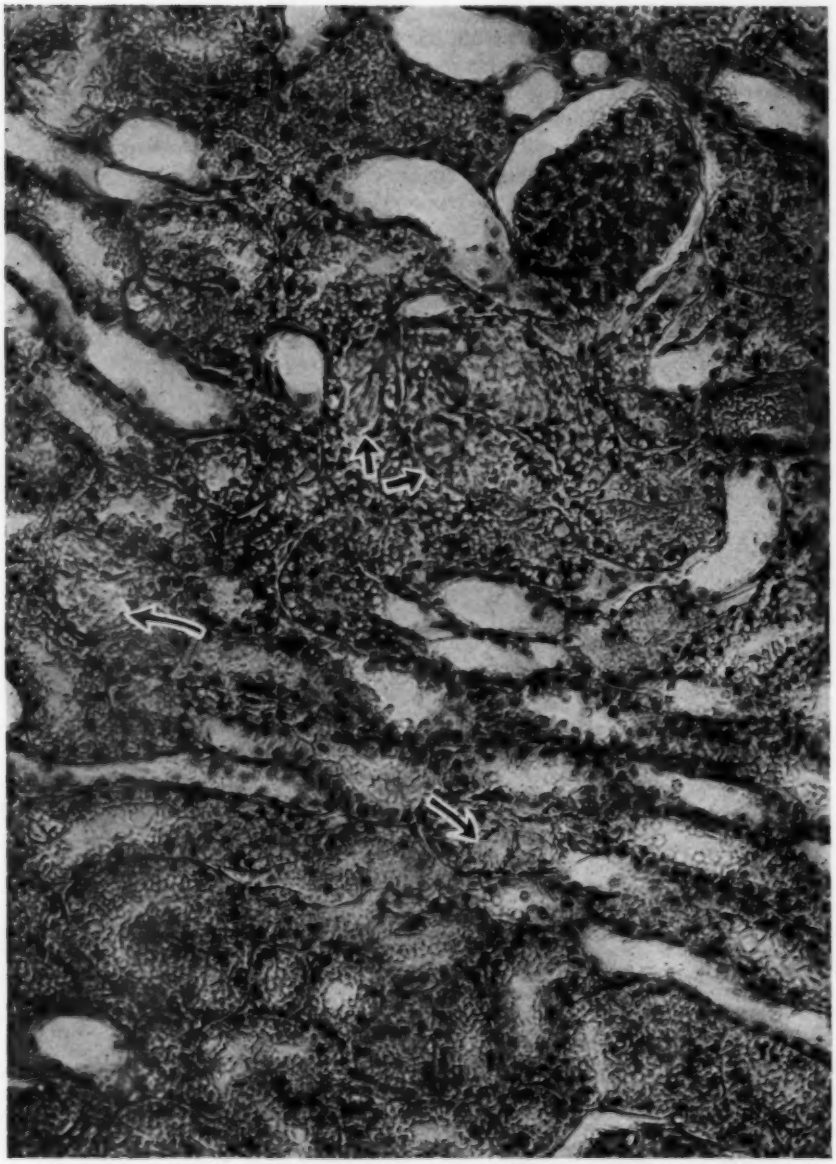

Fig. 2.—Oxalate crystals in the renal tubules of a cat dead one hundred and forty-six hours after oral administration of 3 cc. of 50 per cent ethylene glycol per kilogram of body weight.

Autopsy showed hyperemia and edema of the lungs, concentric dilatation of the heart and cloudy swelling of the kidneys.

Microscopic examination revealed numerous linear crystals in the renal tubules; the tubular epithelium was flat, and its cytoplasm was scanty and hyalinized (fig. 2).

Cat 3.—A male cat weighing 3.3 Kg. was given a dose of 5 cc. per kilogram. The protocol of this animal follows.

Immediately	Salivating
10 min.	Dozing, apathetic, salivating
20 min.	Dozing (salivation stopped)
40 min.	Responsive to smell of food (stands up alert)
45 min.	Dozing, apathetic
23 hr.	Comatose, not responsive to pain or noise, with shallow, rapid respirations and widely dilated pupils
26 hr.	Comatose, with shallow, rapid respirations, dilated pupils reacting weakly to light, brisk reflexes, marked analgesia; cat goes into extensor spasm on being handled
29 hr.	Convulsive; showing spontaneous tonic and clonic convulsions; completely analgesic
31 hr.	Briefly convulsive at infrequent intervals
36 hr.	Deeply comatose; cat died; no autopsy

Cat 4.—A female cat weighing 1.9 Kg. was given a dose of 5 cc. per kilogram. The condition of the animal at intervals after administration of the fluid was as follows:

1 hr.	Apathetic, depressed
4	Markedly ataxic, moderately depressed, slightly analgesic
23	Apathetic, ataxic, analgesic
27	Lying on side, conscious; when stimulated it stands for a moment, then falls; sits only briefly; analgesic
48	Paralyzed, conscious, with fair tendon and corneal reflexes
52	Killed by blow

The observations at autopsy on microscopic examination were essentially the same as those recorded for cat 2.

COMMENT

Ethylene glycol when ingested causes edema of the lungs, dilatation of the heart and a tendency toward bronchopneumonia but leaves its most significant imprint on the kidneys in the form of abundant deposits of oxylate crystals and evidence of degeneration of the epithelium of the convoluted tubules. Clinically its effect is characterized by (*a*) transient stimulation followed by depression of the central nervous system, (*b*) evidence of renal damage, which may proceed to anuria and uremia, (*c*) acute cardiac failure and (*d*) pulmonary edema.

The heavy deposits of crystals in the kidneys suggest that the amount of ethylene glycol which is oxidized to oxalic acid determines its toxicity. It is evident that this opinion and these observations are in conflict with those of some other authors. The discrepancy may be due to their lack of microscopic studies, or to the possibility that oxidation of ethylene glycol is centered in the liver. The latter hypothesis is based on the fact that, taken orally, all of the ingested glycol passes through the liver, whereas when the compound is given intravenously or subcutaneously, the liver is by-passed by a large part of it.

The important role that oxalic acid may play in the toxic effects of ethylene glycol suggests that treatment should be directed toward the relief of oxalate poisoning. Calcium has been recognized as the most effective antidote for oxalate poisoning; an extract of parathyroid gland was proved effective by Kochmann;[10] insulin was found to increase the rate of oxidation of oxalic acid in the body by De Lucia.[11] The desperate state of the person who has ingested any considerable amount of ethylene glycol justifies the use of all of these measures. The tendency toward acute dilatation of the heart and pulmonary edema suggests that great care be used in administering fluid intravenously in any considerable amount or at any but an extremely slow rate.

Experimental studies to determine the effectiveness of the method of treatment indicated should be undertaken with adequate controls and determinations of the blood oxalate level, the urinary oxalate output, the blood calcium and sugar, the blood and urinary p_H and the effect of large volumes of fluid given intravenously on the incidence of pulmonary edema and cardiac failure in animals poisoned with ethylene glycol.

SUMMARY

In man ethylene glycol is a rapidly acting poison; the fatal dose for cats is as low as 1.5 cc. per kilogram (3 cc. per kilogram of antifreeze fluid containing ethylene glycol in the concentration of 50 per cent).

Anatomic evidence suggests that ethylene glycol taken by mouth is rapidly oxidized to oxalic acid. Oxalate crystals are deposited in abundance in the renal tubules.

Death may result from renal failure or acute cardiac failure and pulmonary edema.

Treatment directed at oxalate poisoning is suggested.

Ethylene glycol is poisonous when ingested and the container in which it is sold should be so labeled.

10. Kochmann, M.: Deutsche med. Wchnschr. **60**:406, 1934.
11. De Lucia, P.: Boll. Soc. ital. biol. sper. **4**:756, 1929.

ETIOLOGIC CONCEPTS AND PATHOLOGIC ASPECTS OF AINHUM

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SINCE 1860, when Clark [1] reported on "dry gangrene" occurring in the small toes of Negroes, the condition, later formally described and called ainhum by da Silva Lima,[2] has intrigued clinicians and pathologists. In this communication we shall review the etiologic concepts of this disease and discuss the histologic features of several of our cases.

ETIOLOGY

Theories as to the causation of ainhum are protean, but the etiologic factors comprised in them may be grouped under three general headings: infectious, constitutional and mechanical.

Zambaco pacha [3] was the outstanding proponent of the theory that ainhum was a manifestation of leprosy, but his views were vigorously opposed by Herrick and Earhart,[4] Darling (quoted by Herrick and Earhart [4]), Hektoen,[5] de Brun [6] and others.[7] The treponemes of yaws and syphilis were suggested as causes by Bharucha,[8] Wright [9] and Delanoë.[10] Other writers [11] have considered bacterial causation,

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1. Clark, cited by numerous writers.
2. da Silva Lima, J. F.: Gaz. med. da Bahia **1**:146, 1867; Arch. Dermat. **6**:367, 1880.
3. Zambaco pacha: Tr. Inst. Leprosy Conf. **3**:45, 1897.
4. Herrick, A. B., and Earhart, T. W.: Proc. Canal Zone M. A. **3**:26, 1910-1911.
5. Hektoen, cited by Herrick, J. B.: Philadelphia M. J. **1**:246, 1898.
6. de Brun, H.: Ann. de dermat. et syph. **10**:325, 1899.
7. (*a*) Acton, H. W.: Indian J. M. Research **15**:1085, 1928. (*b*) Friedman, L. J.: Am. J. Roentgenol. **31**:349, 1934.
8. Bharucha, E. S.: Indian M. Gaz. **52**:403, 1917.
9. Wright, L. T.: Urol. & Cutan. Rev. **28**:135, 1924.
10. Delanoë, L.: Bull. Soc. path. exot. **18**:470, 1925.
11. (*a*) Shepherd, F. J.: Am. J. M. Sc. **93**:137, 1887. (*b*) Horwitz, O.: Med. & Surg. Reporter **56**:649, 1887.

Shepherd even predicting that an "ainhum bacillus" might be found which would produce a comparable disease in mice and rabbits, provided the animals inoculated were not white! Davies and Hewer [12] stated that epidermophytosis might produce ainhum, but neither they nor Dschang [13] was able to demonstrate fungi. Parasitologic theories have been advanced, and Wellman [14] wrote at length on the possible role of Sarcopsylla penetrans as a causative factor. Recently Martens and Norris [15] suggested that chronic osteomyelitis may be responsible for the development of ainhum.

Of the constitutional causes, trophoneurosis, that is "some trophic disturbance of the nerve centers," [11a] has appeared most frequently in the literature on this subject.[16] McKnight [17] performed lumbar sympathetic ganglionectomy in a patient with ainhum; when healing occurred he postulated that overactivity of the sympathetic nerves produced vascular spasm resulting in ainhum. Guimaràes [18] stated that the immediate cause was contracture of the arteries but did not specify the basis of that. Anatomic peculiarities of the toes of Negroes have been pointed out as predisposing to vascular embarrassment.[19] Matas,[16e] in considering trophoneurosis as a possible cause, stated that the end result might well be annular scleroderma; others have agreed.[20] Evidence purporting to show that ainhum is but a peculiar manifestation of hyperkeratosis plantare et palmare has been well reviewed by Spencer [21] and ably criticized by Spinzig.[22] The latter held, as do we, that the term "ainhum" is applicable only when the specific lesion of the toe occurs alone and that ainhum should not "be considered a symptom when constrictions occur in association with other diseases. . . . The latter type of constriction may be called 'ainhum-like.'"

Only one reference suggesting dysfunction of the endocrine glands as an etiologic factor was found.[7a]

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12. Davies, J. N. P., and Hewer, T. F.: Tr. Roy. Soc. Trop. Med. & Hyg. **35**:125, 1941.

13. Dschang, Y. D.: Virchows Arch. f. path. Anat. **290**:648, 1933.

14. Wellman, F. C.: J. Trop. Med. **9**:31, 1906; **11**:117, 1908.

15. Martens, V. E., and Norris, R. F.: U. S. Nav. M. Bull. **45**:745, 1945.

16. (*a*) Weinstein, H.: Proc. Canal Zone M. A. **4**:110, 1911-1912. (*b*) Welch, R. S. G.: U. S. Nav. M. Bull. **21**:352, 1924. (*c*) Brayton, N. D.: J. A. M. A. **45**:87, 1905. (*d*) Abbe, T.: M. Rec. **79**:478, 1911. (*e*) Matas, R.: New Orleans M. & S. J. **16**:603, 1888-1889; Tr. Am. S. A. **14**:483, 1896.

17. McKnight, R. B.: North Carolina M. J. **1**:76, 1940.

18. Guimaràes, cited by Dell'Orto, J.: New Orleans M. & S. J. **8**:516, 1880-1881.

19. Ashley-Emile, L. E.: J. Trop. Med. **8**:33, 1905.

20. Messum, G.: Lancet **1**:932, 1891. McKnight.[17] Guimaràes.[18] Ashley-Emile.[19]

21. Spencer, H. A.: South African M. Rec. **5**:103, 1907.

22. Spinzig, E. W.: Am. J. Roentgenol. **42**:246, 1939.

That the disease is familial is accepted by many. Horwitz and Tunick,[23] Duhring [24] and others [25] have observed the lesion in several members of the same family. Relationship to other Negro tendencies, such as that toward keloids, has not been definitely established, but several investigators considered that ainhum may bear some relation to the "fibrogenetic" tendency of the race.[26] Despite a statement to the contrary,[16c] in utero development of ainhum has not been observed; congenital spontaneous amputations should not be confused with ainhum.

The final group of theories concerns mechanical factors. The little toe, because of its position, may be especially liable to injury,[27] to minute abrasions by jungle grasses and the like. As was indicated in a clinical communication,[26] this can hardly be the sole explanation. On the Isthmus of Panama the 45 patients exhibiting ainhum were exclusively Negroes, whereas Panamanian mestizos, who wear shoes with even less frequency than the Negroes, seemed to be free of the disease. The wearing of toe rings or the application of ligatures is not responsible although, as Wile pointed out, the appearance of the lesions strongly suggests such mechanical causes.[24]

PATHOLOGY

The first studies on the pathologic anatomy of ainhum were made by Wücherer [28] at the request of da Sillva Lima, in Bahia, Brazil. The former sent material to Weber in London, England, where De Morgan and Wood first reported in 1867,[25b] and to von Schüppel, of Tübingen, Germany, who published a report in 1872.[29] Following these early reports, many excellent descriptive studies have appeared,[30] but little new has been added.

During the past several years we have had the opportunity to review the records of 45 persons suffering from ainhum on the Isthmus of Panama and to observe a dozen clinically. We have been able to study histologic material in 6 cases, 1 of which is illustrated in the accompanying figures. The descriptions based on these data conform to a considerable degree with previous reports, but our interpretations may be at some variance with those of other workers.

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23. Horwitz, M. T., and Tunick, I.: Arch. Dermat. & Syph. **36**:1058, 1937.

24. Duhring, L. A.: Am. J. M. Sc. **87**:150, 1884.

25. (*a*) Dupouy, E.: Arch. de méd. nav. **41**:260, 1884. (*b*) Weber, H.: Tr. Path. Soc. London **18**:277, 1867. (*c*) Wheatland, M. F.: J. A. M. A. **45**:631, 1905. (*d*) Doyle, E. A. G.: Brit. M. J. **1**:1346, 1889. (*e*) Simon, K. M. B.: J. A. M. A. **76**:590, 1921. (*f*) Weinstein.[16a]

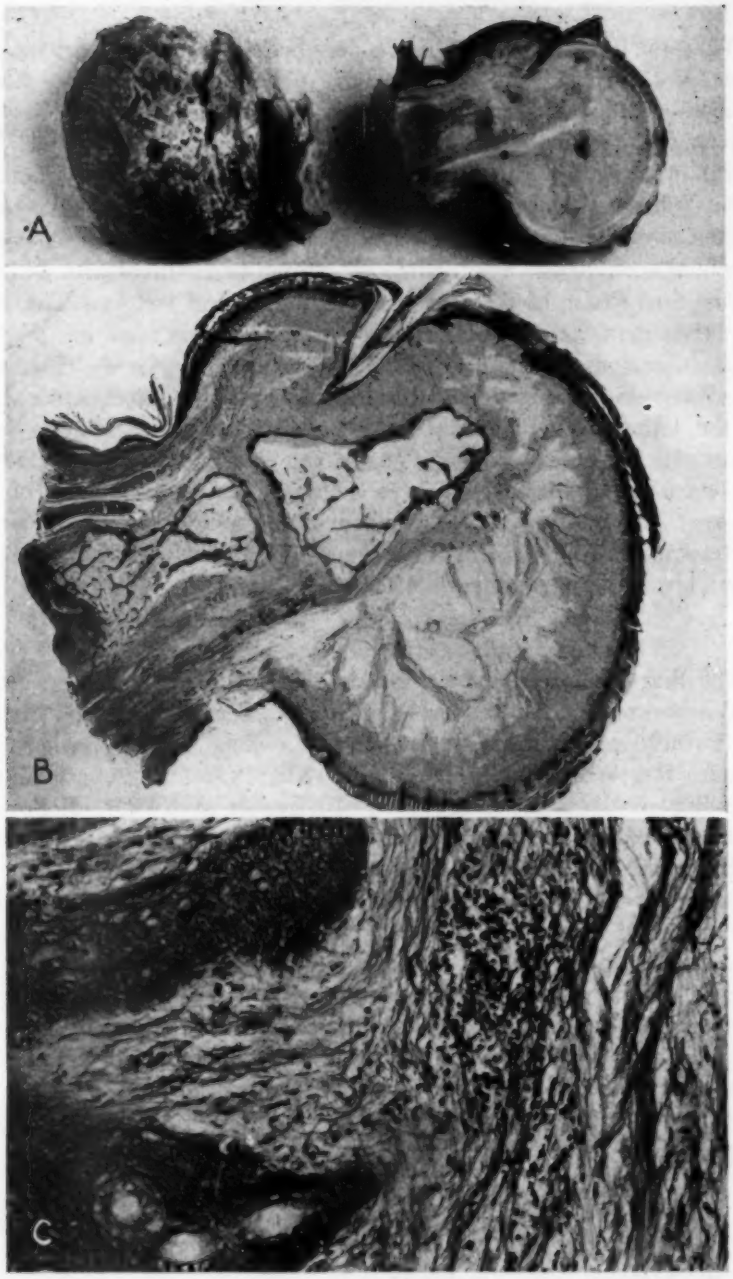
26. Kean, B. H.; Tucker, H. A., and Miller, W. C.: Tr. Roy. Soc. Trop. Med. & Hyg. **39**:331, 1946.

27. Eyles, C. H.: Lancet **2**:576, 1886.

28. Wücherer, O.: Virchows Arch. f. path. Anat. **56**:374, 1872.

29. von Schüppel, F. G.: Virchows Arch. f. path. Anat. **56**:381, 1872.

30. Davies and Hewer.[12] Dschang.[13] Weinstein.[16a] Duhring.[24] Eyles.[27]



Case 1. *A*, bisected toe with ainhum showing the typical "groove"; a moderately advanced lesion; × 2. *B*, microtessar photograph of a section of the toe with ainhum; × 4. Periostitis of significant degree was not seen microscopically. *C*, low grade inflammatory changes present in the corium and the subcutaneous tissues; × 125. These changes seemed to be no more marked at the site of the furrow than in other portions of the sections studied. (Mr. Julius Weber, Department of Photography, Columbia University College of Physicians and Surgeons, took photograph designated *C*.)

The disease in our material was limited to Negro males, and the little toe was the only digit involved. At the level of the digitoplantar fold was a deep encircling groove or furrow separating the toe from the remainder of the tarsus. The toe itself was bulbous, often swollen and frequently painful. The nail was atrophied. When advanced, the "constriction" had progressed until a thin pedicle kept the small potato–like toe attached to the foot. (Excellent photographs of the lesion are available in most of the standard textbooks of tropical medicine.) In our last 3 cases the toe was amputated *proximal* to the groove so that the site of "constriction" could be adequately studied.

In microscopic sections the epithelium of the toe showed moderately severe hyperkeratosis, some parakeratosis and slight to moderate irregular acanthosis. Surprisingly enough, however, these changes were only slightly more intense at the site of the groove than either distally or proximally. Within the superficial and the deeper layers of the corium and in the subcutaneous tissue was a slight to moderate chronic inflammatory reaction with infiltrating lymphocytes and plasma cells (*C* in figure). Occasionally neutrophils and giant cells of the chronic inflammatory reaction type could be seen. Fibrosis generally was moderate and assumed no pattern which conceivably could cause the "constricting band" which has figured so conspicuously in some previous descriptions.[31] In some areas the fibrous tissue was hyalinized; in others it appeared fragmented. The bone was atrophied and had generally disappeared at the site of the groove. Osteoclasts could occasionally be identified. The walls of the blood vessels were thick and the lumens narrow, and infrequently the intimal layer showed proliferation. The nerves on the whole were not remarkable, although some perineural fibrosis could be identified. The glands generally appeared normal, although often widely separated by fibrous tissue. Much fat was generally present in the subcutaneous tissue of the ventral aspect of the toe.

The foregoing description conforms rather monotonously to the many previously published reports on ainhum. However, we believe undue significance has been placed on various histologic features:

(*a*) The groove or furrow at the digitoplantar fold is not caused by a *constricting band* of fibrous tissue.

(*b*) The epithelial changes are not striking and identical changes may be seen in the toes of those who do not have ainhum.

(*c*) The thick walls of the blood vessels and the occasionally proliferated intima are matched in controls.

(*d*) The changes in nerves and glands do not appear significant.

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31. Tye, M.: New England J. Med. **234**:152, 1946. Gray and Blache, cited by Spinzig.[22] Acton.[7a] Friedman.[7b] Davies and Hewer.[12] Duhring.[24]

There remain the atrophy of bone and the subcutaneous inflammatory reaction and fibrosis, changes which seem significant but are difficult to evaluate. The changes in the bones have been of great interest for many years, especially from a roentgenologic standpoint.[32] Recently Martens and Norris,[15] reporting a case of ainhum, stated "the essential lesion appears to be a chronic osteomyelitis" and that "the fibrous constriction of the little toe is not the cause of bone destruction but is the result of cicatrization of previously inflamed tissue." Incidentally, they sectioned toes of patients without ainhum and noted thick-walled vessels like those often described as of significance in ainhum, a finding which is mentioned also by Ash and Spitz [33] and which we have noted in sections of the toes of our controls.

In our material the evidence that chronic osteomyelitis is the essential lesion of ainhum has not been impressive. More suggestive have been the low grade cellulitis and fibrosis of the corium. In 44 of the 45 patients on the Isthmus of Panama pain was a symptom sometime during the course of the disease. In several patients cellulitis was so prominent that amputation was delayed for fear of spreading the infection. From 2 patients recently observed, pure cultures of Staphylococcus aureus (hemolytic) were obtained when the area of "constriction" was incised and pus released. It is, of course, possible that this infection may be secondary, occurring in a locus minoris resistentiae. In sections available for study, however, the inflammatory reaction is not extensive, and it is difficult to conceive how it could produce the lesion which is so distinctive grossly and so difficult to define microscopically.

SUMMARY

In summary, the cause of ainhum is unknown, and its pathogenesis is not clear. The racial factor seems most important.

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Harold A. Tucker, The Johns Hopkins Hospital, Department of Medicine. Baltimore, Md.

32. Herrick and Earhart.[4] Friedman.[7b]

33. Ash, J. E., and Spitz, S.: An Atlas of Pathology of Tropical Diseases. Philadelphia, W. B. Saunders Company, 1945, p. 344.

PRIMARY CYSTIC TUMOR OF THE DIAPHRAGM

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THE first report of a primary tumor (fibroma) of the diaphragm was made by Granchi [1] in 1868. The paucity of similar reports since then indicates the rarity of primary diaphragmatic neoplasms. In 1944 Robson and Collis,[2] in summarizing the data on the recorded ones, were able to collect 21 benign and cancerous tumors primary in the diaphragm and added a benign one observed by them. In a recent review of this subject we have found a total of 32 reported benign and cancerous diaphragmatic tumors. This series includes 4 the diagnosis of which was not confirmed by operation or autopsy.[3] It is anticipated,

TABLE 1.—*Noncancerous Diaphragmatic Tumors*

	Author	Year	Sex	Age	Side	Type	Symptoms
1.	Granchi [1]	1868	M	..	..	Fibroma	Not described
2.	Clarke [9]	1887	F	65	R	Lipoma	None
3.	Kramer [11]	1899	M	54	R	Chondroma	None
4.	Bonamy [10a]	1912	F	34	R	Multiple fibroma	Swelling; no pain
5.	Burvill-Holmes and Brody [12]	1932	M	73	R	Angiofibroma	None
6.	Spangenberg, Gattini and Sloer [3c]	1935	M	73	R	Unknown	Not stated
7.	Gravano [3b]	1935	..	..	..	Unknown	.....
8.	Soderlund [9b]	1937	F	50	L	Lipoma	Pain in chest
9.	Ballon and Spector [9c]	1939	F	45	L	Lipoma	None
10.	Kinsella [16]	1939	F	62	R	Fibromyoma	Mass between ribs; unrelated pain
11.	Butler [17a]	1939	F	34	L	Cyst (type?)	Pain in chest
12.	Nylander [13]	1942	M	17	R	Lymphangioma	Abdominal pain
13.	Arkless [15]	1942	M	38	L	Rhabdomyofibroma	Pain in chest; cough
14.	Denis [3a]	1942	F	20	R	Unknown	Pain in chest
15.	Ackerman [3d]	1942	M	48	R	Unknown	Pain in chest
16.	Soto [9d]	1943	M	14	L	Lipoma	Pain in chest
17.	Robson and Collis [2]	1944	M	22	R	Cyst (type?)	Pain in chest; dyspnea
18.	Klassen, Patton and Bemen [14]	1945	M	42	R	Neurofibroma	Pain in chest
19.	Scott and Morton (Present report)	1946	M	29	L	Cyst (type?)	Pain in chest

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This study was facilitated by a grant from Mr. Hill Blackett, Chicago.

1. Granchi, M.: Bull. Soc. anat. de Paris **43**:385, 1868.

2. Robson, K., and Collis, J. L.: Brit. J. Tuberc. **38**:3, 1944.

3. (*a*) Denis, R., and Garre, O.: Prensa méd. argent. **29**:380, 1942. (*b*) Gravano, L.: Semana méd. **2**:705, 1935. (*c*) Spangenberg, J. J.; Gattini, H., and Sloer, M.: Prensa méd. argent. **22**:17, 1935. (*d*) Ackerman, H. J.: Am. J. Roentgenol. **47**:711, 1942.

however, that with the increasing use of routine fluoroscopy and roentgenography of the chest diaphragmatic tumors will be found with increasing frequency. Reports of tumors are summarized in tables 1 and 2, including the one reported here.

REPORT OF TUMOR

G. H., a 29 year old white man, was first admitted to the University of Chicago Clinics (service of Dr. A. Brunschwig) on Aug. 19, 1945, at which time he stated that two years previously he had consulted a physician because of poor appetite, loss of weight and fatigue. At that time a diagnosis of pulmonary tuberculosis was made on the basis of roentgenograms of the chest and "positive sputum."

TABLE 2.—*Primary Diaphragmatic Cancers*

	Author	Year	Sex	Age	Side	Type	Symptoms
1.	Dalzell [6]	1887	F	42	R	Round cell sarcoma	Cough
2.	Alexander [4a]	1896	..	..	..	Fibrosarcoma	None described
3.	Gross [4b]	1911	M	30	L	Fibrosarcoma	Pain in chest
4.	Sauerbruch [4]	1913	F	43	L	Fibromyosarcoma	Abdominal pain
5.	Van Nes [4c]	1921	F	65	L	Fibrosarcoma	Abdominal pain
6.	Muller [5b]	1933	F	45	R	Myosarcoma	None
7.	Kirschbaum [5c]	1935	M	58	L	Leiomyosarcoma	Palpable mass
8.	Kirschbaum [5c]	1935	M	47	R	Rhabdomyosarcoma	Thoracic pain, cough, hemoptysis
9.	Ryan [5d]	1939	..	..	..	Myosarcoma	None described
10.	Peery and Smith [5e]	1939	M	14	R	Rhabdomyosarcoma	Pain in chest; dyspnea
11.	Gale and Edwards [8]	1939	M	54	R	Carcinoma of liver from embryonal rest	Pain in shoulder
12.	Petacci [7]	1940	M	45	R	Undifferentiated sarcoma	Pain in chest
13.	Hyman and Lederer [4d]	1941	F	73	R	Fibrosarcoma	Edema of legs
14.	Ackerman [5f]	1942	M	27	R	Fibrosarcoma	Pain; cough

Eight subsequent examinations of his sputum gave negative results. Subsequently he spent three weeks in a sanatorium, where after a thorough examination he was informed that he did not have tuberculosis but a cyst of the left lung. At about this time he began to note a sharp, nonradiating pain at the left costal margin on deep inspiration or on bending or lifting.

For two or three years prior to his admission he noted a mild productive cough, but the sputum was not purulent, and no hemoptysis occurred. So far as he knew he had not been in contact with a tuberculous person. Although his weight fluctuated considerably, he was usually underweight, and one month before admission he was rejected for military duty because he was "underweight and neurotic."

On admission the examination gave essentially negative results. The blood hemoglobin content was 16.2 Gm.; the red blood cell count, 4,590,000; the white cell count, 7,800; the Kahn test, negative. There were no abnormal urinary findings. Roentgenograms of the chest revealed, near the costophrenic angle, a rounded protrusion from the left leaf of the diaphragm, which measured 3 by 6 cm. in diameter (fig. 1 *A*). Along the inferior medial aspect of the mass a shell of calcification was noted. At fluoroscopy the rounded mass was seen to move freely with the left leaf of the diaphragm. Roentgenograms of the stomach and

duodenum showed no relationship between these organs and the mass in the left side of the diaphragm. The Casoni test (intradermal injection of echinococcus antigen) was negative.

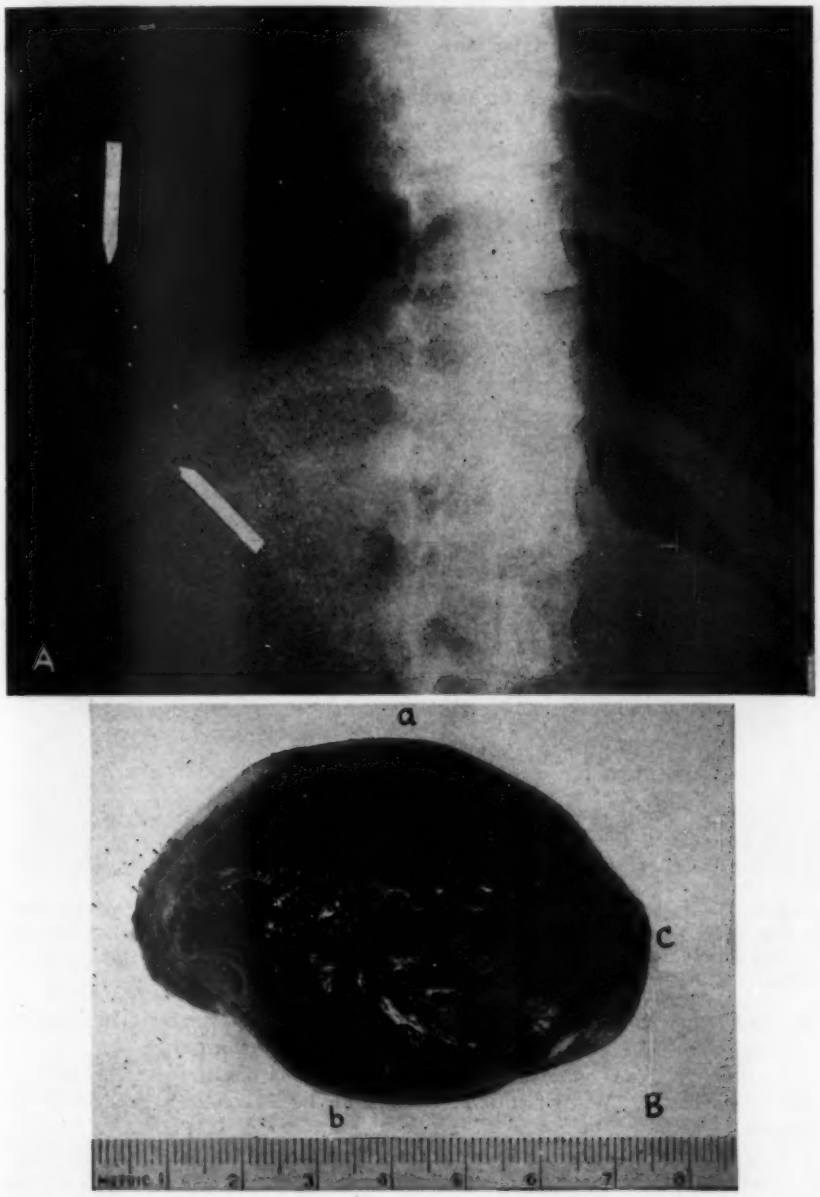

Fig. 1.—*A*, roentgenogram of the lower part of the chest showing a mass in the left leaf of the diaphragm with a calcified medial wall. *B*, intact specimen showing (*a*) the pleural surface above, (*b*) the peritoneal surface below and (*c*) the surrounding diaphragmatic muscle.

On August 23 the patient was anesthetized with nitrous oxide, ethylene and ether, and the abdomen was opened by a left paracostal incision. An exploration of the abdominal viscera showed no abnormality. Embedded in the central portion of the dome of the left leaf of the diaphragm was a cystic structure, measuring about 6 cm. in diameter, from which a few fibrous adhesions extended to the upper pole of the spleen. The latter was mobilized except for its pedicle and retracted downward. Attempts were made to enucleate the tumor from the diaphragm, but it was apparent that if this was done the danger of breaking the tumor would be great; therefore, it was excised by sharp dissection of the adjacent diaphragmatic musculature. A defect about 10 by 8 cm. remained in

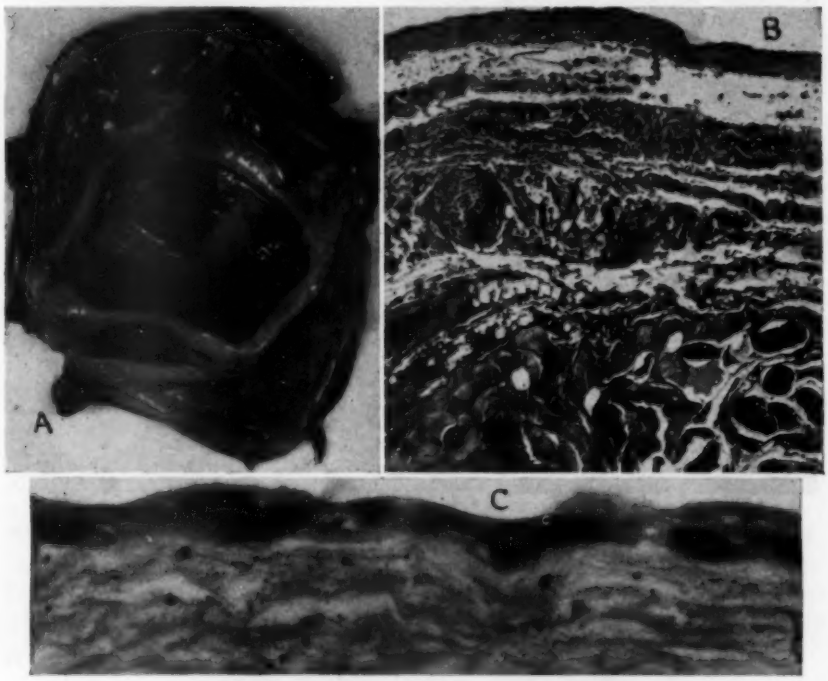

Fig. 2.—*A*, opened specimen demonstrating cystic character. *B*, photomicrograph (× 65) of entire cyst wall, including adjacent diaphragmatic muscle. *C*, photomicrograph (× 1050) of cyst wall to show the character of the epithelial lining.

the diaphragm, and this was closed with interrupted silk sutures. In order to facilitate closure of the diaphragm, splenectomy was performed.

Convalescence was uneventful, and the patient was discharged from the hospital September 2. When last seen, October 31, he was entirely relieved of his symptoms.

The specimen consisted of an ovoid cystic structure which measured 7 by 5 by 4 cm. and weighed 78 Gm. It was bordered on the upper surface by pleura and on the lower surface by peritoneum. About the greater circumference was a thick fringe of incised musculature (fig. 1 *B*). When the specimen was incised, a cystic cavity was entered containing a chocolate-colored fluid in which cholesterol crystals were dispersed. The cyst wall resembled dense fibrous tissue and varied from 1 to 4 mm. in thickness. The inner surface was coarsely trabeculated, and

the outer surface was firmly attached to the surrounding muscle. Throughout the wall, but especially in the lower medial part, and in a somewhat trabecular pattern, were areas of an extremely hard tissue, which cut as though calcified.

The excised spleen was normal in size and shape and weighed 238 Gm.

Microscopic examination of the wall of the diaphragmatic cyst (fig. 2) showed it to be lined with a single to double layer of flat to cuboidal epithelial cells. Beneath this were densely packed strands of fibrous and collagenous connective tissue. In some areas the fibrous tissue was calcified. Throughout the wall there were irregularly scattered foci of lymphocytes, and near its outer margin, which blended into the surrounding striated muscle, were a few areas of loosely arranged connective tissue containing numerous small vessels. Microscopic examination of the cyst fluid revealed typical cholesterol crystals.

Diagnosis: primary cyst of the diaphragm; origin undetermined.

COMMENT

As stated, 14 cancerous and 19 noncancerous primary diaphragmatic tumors have been encountered since 1868. Of the cancers, 5 were thought to be fibrosarcoma.[4] Hyman,[4d] however, is not in agreement with the diagnosis of the one reported by Alexander.[4a] Six tumors were thought to originate from muscle.[5] Two of these have been termed rhabdomyosarcoma; 2, myosarcoma; 1 leiomyosarcoma, and 1, fibromyosarcoma. Dalzell[6] reported a round cell sarcoma and Petacci[7] an undifferentiated sarcoma. The tumor reported by Gale and Edwards[8] is unique in that it was an epithelial growth distinctly located in the diaphragm and apparently originating from an embryonic rest of liver tissue. The noncancerous tumors include 4 diagnosed as lipoma,[9] 2 as fibroma,[10] 1 as chondroma,[11] 1 as angiofibroma,[12] 1 as lymphangioma,[13]

4. (*a*) Alexander, B., cited by Hyman and Lederer.[4d] (*b*) Gross, H.: Deutsche Ztschr. f. Chir. **109**:425, 1911. (*c*) Van Nes, C. P.: Nederl. tijdschr. v. geneesk. **65**:583, 1921. (*d*) Hyman, M. A., and Lederer, M.: Arch. Path. **31**: 204, 1941. (*e*) Ackerman.[3d]

5. (*a*) Sauerbruch, F., and O'Shaughnessy, L.: Thoracic Surgery: Revised Edition of E. F. Sauerbruch's "Die Chirurgie der Brustorgane," Baltimore, Edward Arnold & Company, 1937. (*b*) Muller, W.: Centralbl. f. allg. Path. u. path. Anat. **58**:353, 1933. (*c*) Kirschbaum, J. D.: Am. J. Cancer **25**:730, 1935. (*d*) Ryan, E. J., cited by Robson and Collis.[2] (*e*) Peery, T. M., and Smith, W. A.: Am. J. Cancer **35**:416, 1939.

6. Dalzell, cited by Gale and Edwards.[8]

7. Petacci, M.: Policlinico (sez. chir.) **47**:136, 1940.

8. Gale, J. W., and Edwards, S. M.: J. Thoracic Surg. **9**:185, 1939.

9. (*a*) Clarke, F. W.: Tr. Path. Soc. London **38**:324, 1887. (*b*) Soderlund, G.: Acta radiol. **18**:388, 1937. (*c*) Ballon, H. C., and Spector, L.: Canad. M. A. J. **41**:487, 1939. (*d*) Soto, M. V.: J. Internat. Coll. Surg. **6**:146, 1943.

10. (*a*) Bonamy, R: Paris chir. **4**:1051, 1912. (*b*) Granchi.[1]

11. Kramer, S. P.: Virchows Arch. f. path. Anat. **156**:188, 1899.

12. Burvill-Holmes, E., and Brody, W.: Am. J. M. Sc. **183**:679, 1932.

13. Nylander, P. E. A.: Zentralbl. f. Chir. **69**:929, 1942.

1 as neurofibroma,[14] 1 as rhabdomyofibroma,[15] 1 as fibromyoma[16] and 3 as cysts.[17] Four tumors in regard to which a final diagnosis of diaphragmatic tumor could not be made are included in the tabulation of benign tumors.[3]

These neoplasms were found in patients varying from 14 to 73 years of age, with an average age of 43. Of the 28 tumors whose locations were stated, 18 were in the right leaf and 10 were in the left leaf of the diaphragm. Eighteen of the tumors were in males and 12 in females. With regard to 2 the sex of the patient was not stated.

Primarly diaphragmatic tumors did not always produce symptoms, and when the latter were present they followed no specific pattern. The most constant symptom noted was pain in the chest, also in the hypochondrium and occasionally radiating along the course of the regional intercostal nerves or to the shoulder. Cough was occasionally a complaint and hemoptysis once. Bulging intercostal masses, as well as masses in the right and left upper quadrants of the abdomen have been noted. Dyspnea is sometimes present, and pleural effusion may occur.

The clinical diagnosis can hardly be made on the basis of the history and the results of the physical examination, owing to the rarity of the condition; it is finally made on roentgenographic evidence, and it is possible to differentiate between abdominal and intrathoracic masses by roentgen study.[3d] Intrathoracic tumors touching the diaphragm show motion synchronous with the normal respiratory excursion of the diaphragm. If the tumor lies underneath the diaphragm and protrudes through it into the thoracic cavity, a paradoxic movement of the tumor's shadow is noted similar to that characteristic of diaphragmatic hernia. The paradoxic movement of a diaphragmatic hernia may not be demonstrable in the presence of adhesions within the hernial contents. Diagnostic pneumothorax is useful in differentiating lesions occurring in the base of the lung. Pneumoperitoneum may aid in outlining the position of the tumor. Roentgenographic examination of the gastrointestinal tract is of value in differentiating lesions involving the stomach or the bowel. When cystic tumors with calcified walls are encountered, intradermal or complement fixation tests are indicated to rule out echinococcus cysts.

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14. Klassen, K. P.; Patton, R., and Bemen, F.: J. Thoracic Surg. **14**:407, 1945.

15. Arkless, H. A.: M. Bull. Vet. Admin. **19**:225, 1942.

16. Kinsella, T. J., in discussion on Gale and Edwards.[8]

17. (*a*) Butler, E. F., in discussion on Gale and Edwards.[8] (*b*) Robson and Collis.[2]

DEVELOPMENT OF SEBACEOUS GLANDS FROM INTRALOBULAR DUCTS OF THE PAROTID GLAND

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AS GRUENWALD has recently pointed out, the lability of determination and presence of latent potencies of cells are more common than was originally believed, and morphologic determinations which were thought to be fixed could be changed under experimental conditions, such as tissue culture and regeneration.[1] It is difficult, however, to obtain an accurate knowledge of the different prospective potencies of the cells of the various tissues, as the finding of the appropriate developmental stimulus for obtaining certain differentiations depends more or less on chance. In human pathology, accidental findings may sometimes bring to light unsuspected potencies of certain cells or prove the reality of potencies whose presence had been assumed on theoretic grounds. Of this the following case is a good example.

REPORT OF A CASE

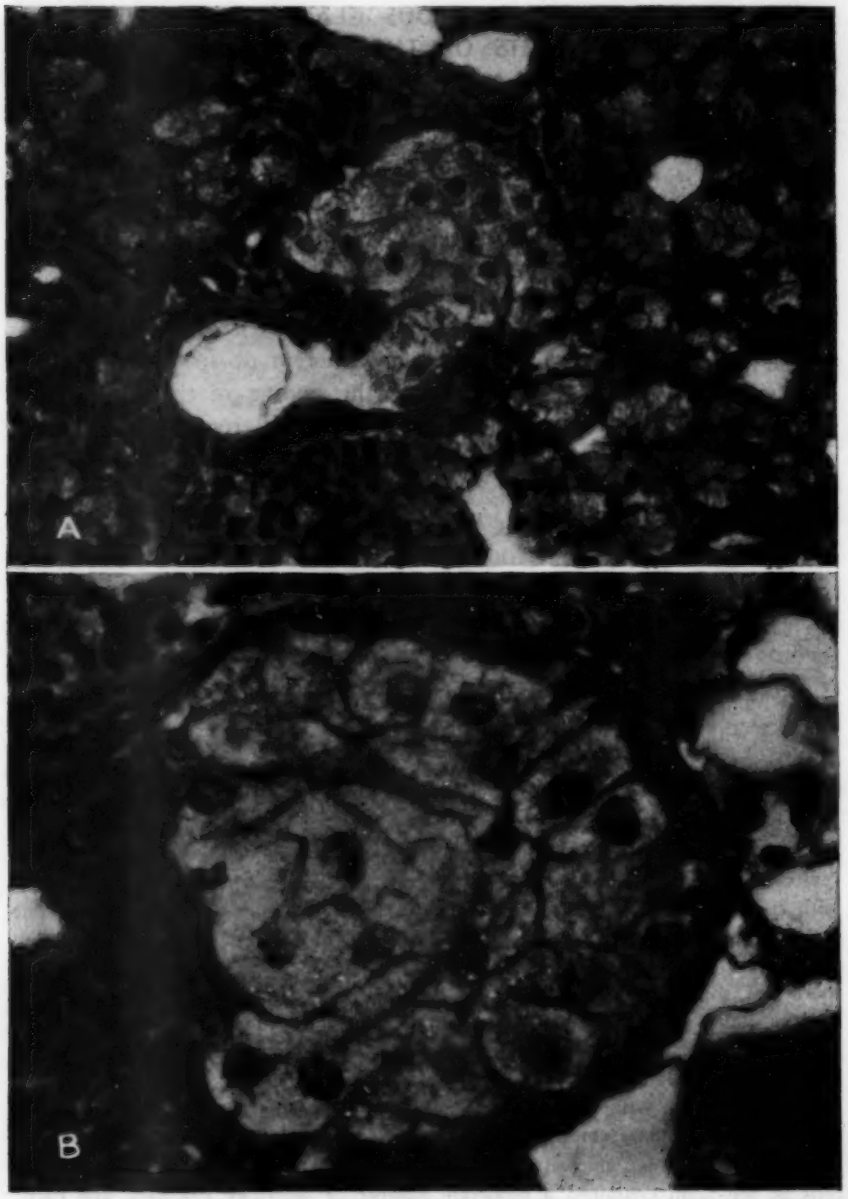
A white woman 34 years old had been operated on elsewhere for a tumor of the left parotid gland. Examination showed it to be a noncancerous mixed tumor. There was a local recurrence of the growth, slowly growing, which was removed with the surrounding glandular tissue (Dr. M. J. Hugenholtz). At this moment, two and a half years after operation, the patient is without recurrence.

Microscopically, the tumor was a typical mixed tumor of the parotid gland, consisting of strands and nests of small epithelial cells with thin intercellular bridges and several glandular cavities, lying in a myxomatous stroma which in several places resembled cartilage. There was sometimes squamous metaplasia with cornification. In the stroma lay large cells with long, irregular processes. Only after a prolonged search could an isolated mitosis be found in the tumor cells. The tumor was generally well encapsulated. In a few places, however, there was some infiltration of the capsule by tumor tissue. The lobules of the parotid gland immediately bordering on the tumor capsule showed atrophy and partial disappearance of the acini and some lymphocytic infiltration. There were numerous intercalated ducts, the protoplasm of which sometimes contained small granules staining with aniline blue. Here and there a mitotic division was observed in the ductal epithelium.

The other lobules showed the normal structure of the parotid gland. The cells of the acini contained a moderate number of secretory granules, and each had a narrow rim of chromophil substance. In general, the striated and inter-

1. Gruenwald, P.: Arch. Path. **36**:190, 1943.

calated ducts showed nothing particular except a few small ducts lined by mucous cells. In several otherwise normal lobules there were epithelial structures which did not belong to the normal components of the parotid gland. They varied



A, sebaceous gland developing from a small duct; × 280. There is holocrine secretion. *B*, higher magnification to show the flattened cells at the periphery and the large nuclei of the sebaceous gland; × 580.

much in size; the diameter of the larger ones was as much as 400 microns. They were surrounded by a thin basement membrane. They were partly solid and consisted of epithelial cells, the outermost layer being flattened, with fairly dark-staining protoplasm, whereas the more centrally placed cells contained protoplasm which showed fine progressive vacuolation. It did not contain chromophil substance or secretory granules. The nuclei were larger than those of the acinous cells; they contained less chromatin and a distinct nucleolus. In the central cells the nuclei were sometimes irregularly indented by the vacuoles and pyknotic.

Serial sections showed that these structures always originated from ducts, either striated or intercalated ducts. The epithelium of the ducts transformed into flattened cells became multilayered and gave origin to the structures described. The transformation of the ductal epithelium was fairly sudden. The impression was gained that the abnormal epithelial formations did not develop in the continuity of the ducts but in short branches which ended blindly. There was sometimes typical holocrine secretion. Mitotic divisions were only rarely observed.

COMMENT

It follows from the foregoing description and the photomicrographs that the epithelial formations could not be distinguished from small sebaceous glands. By their nuclear structure, by the progressive vacuolation of their cytoplasm and by the absence of chromophil substance and secretory granules they could easily be differentiated from the acinous cells of the parotid gland.

It could be proved that the sebaceous glands developed from intralobular ducts, striated or intercalated. Unfortunately, it was impossible to determine when this development had started, so that it remains unknown whether they were present before the tumor and the first operation or whether these abnormal circumstances were the stimulus for the peculiar differentiation of the ductal epithelium.

Theoretically, the development of sebaceous glands from the ducts of the parotid gland cannot be considered as strange, though the literature disclosed no instance and only 1 case of mixed tumor of the parotid gland containing sebaceous glands.[2] The parotid gland develops from the ectoderm on the deep aspect of the cheek,[3] and it is also known that during puberty sebaceous glands develop in this region.[4] The case

2. Harvey, W. F.; Dawson, E. K., and Innes, J. R. M.: Debatable Tumours in Human and Animal Pathology, London, Oliver & Boyd, Ltd., 1940, p. 24, fig. 25.

3. Hamilton, W. J.; Boyd, J. D., and Mossman, H. W.: Human Embryology, Baltimore, Williams & Wilkins Company, 1945, p. 161.

4. Weatherford, H. L.: A Textbook of Histology, Philadelphia, The Blakiston Company, 1944, p. 319.

which I have described shows therefore a prospective potency of the ductal epithelium of the parotid gland which could be presumed on theoretic grounds.

SUMMARY

In the normal lobules of a parotid gland showing a recurrence of a typical mixed tumor were found small sebaceous glands developing from striated or intercalated ducts.

Case Reports

LEIOMYOSARCOMA INVOLVING THE RIGHT URETER

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LEIOMYOSARCOMA is not a common tumor. There seems to be no distinct characteristic clinical picture, so that the final diagnosis must await microscopic study. The tumor is highly vascularized, has little stroma and is studded with mitotic figures. Its cells are spindle-shaped, unstriated muscle type with large deeply staining nuclei. It seems that any organ having unstriated muscle fibers may become the site of development of leiomyosarcoma. In looking over the literature we have been unable to find a report of a case of leiomyosarcoma similar in site to the one to be recorded later in this presentation.

Krauskopf [1] reported an analysis of 30 cases, to which he added his own. The sites were as follows: pleura, foot and vulva (1 case each); urinary tract (5 cases—the growth involving the bladder in 3 and the kidneys in 2); gastrointestinal tract (7—the growth involving the cecum, the ileum and the liver in 1 case each and the stomach and the jejunum in 2 cases each); uterus (16 cases). In a review of the literature on smooth muscle tumors of the stomach Chaffin [2] collected 363 cases, 16 of which were definitely cases of leiomyosarcoma. Concerning 2 of the 16, no data were recorded as to sex and age, and therefore these 2 could not be used in our table 1. Since the publication of Chaffin's review Baumgartner [3] has reported 1 case and Horsley and Berger [4] 3 cases of leiomyosarcoma of the stomach. Hallock, Watson and Berman [5] reported an interesting case in which the tumor arose from the subdiaphragmatic portion of the inferior vena cava. To this group we add a case in which retroperitoneal leiomyosarcoma was densely adherent to the right uterer.

Table 1 reveals that the frequently heard statement that sarcoma occurs principally in the young and carcinoma in the middle-aged and the aged is not valid so far as leiomyosarcoma is concerned. It might best be said that cancer is a disease principally of middle life but may occur at any period of life. In regard to sex incidence, table 1 cannot be used as a criterion, because included in it are 16 cases of uterine and 1 case of vulvar leiomyosarcoma. Table 2 provides a

1. Krauskopf, H.: Am. J. Surg. **22**:192, 1933.
2. Chaffin, L.: West. J. Surg. **46**:513, 1938.
3. Baumgartner, C. J.: West. J. Surg. **47**:27, 1939.
4. Horsley, G. D., and Berger, R. A.: Ann. Surg. **112**:22, 1940.
5. Hallock, P.; Watson, C. J., and Berman, L.: Arch. Int. Med. **66**:50, 1940.

better basis for judgment since only cases of leiomyosarcoma of the stomach are included. It will be noted that 65 per cent of the patients were females and 35 per cent were males. This is a ratio of almost 2:1 favoring females. It is contrary to the report by Horsley and Berger,[4] who stated: "There are no sex differences, the occurrence being about equal in males and females." Andersen and Doob,[6] while reporting a

TABLE 1.—*Sex and Age Incidence of Leiomyosarcoma*

Authors	Sex and Age, Yr.	
	Female	Male
Baumgartner [3]	..	39
Hallock and others [5]	31	..
Horsley and Berger [4]	62	..
	48	..
	..	34
Chaffin [2]	47	..
	43	..
	17	..
	68	..
	56	..
	65	..
	40	..
	32	..
	32	..
	34	..
	..	39
	..	40
	..	41
	..	33
Krauskopf [1]	53	..
	60	..
	26	..
	40	..
	47	..
	44	..
	47	..
	46	..
	50	..
	38	..
	52	..
	55	..
	55	..
	40	..
	31	..
	39	..
	45	..
	56	..
	51	..
	21	..
	50	..
	50	..
	83	..
	48	..
	46	..
	..	4
	..	65
	..	50
	..	19
	..	35
	..	40
Rossien and Russell: Arch. Path., this issue	55	..
Number of each sex	39	12
Average age	41.23	36.58
Age span	17 to 83	4 to 65

case of leiomyosarcoma of the duodenum, mentioned 18 previously reported similar cases. They stated that, "of the 18 patients whose sex was specified, 12 were men." Their patient was also a male. Hence, their findings would, off hand, appear to be a reversal of our conclusion. However, the 5 cases of leiomyosarcoma of the urinary

6. Andersen, D. H., and Doob, E. F.: Arch. Path. **16**:795, 1933.

tract included in Krauskopf's [1] report were distributed between the sexes in a ratio of 3:2 favoring females. Further, deducting from table 1 the 17 cases in which the female genitals were involved, one still notes that 64.7 per cent of the patients with leiomyosarcoma are females and 35.3 per cent are males.

REPORT OF CASE

Sept. 9, 1941 a white housewife aged 55 years reported for study and gave the following history:

About three months before, a burning sensation devloped in the right lower abdominal quadrant and in the loin. At the onset the pain occurred immediately after meals and at three to four hour intervals. However, after a few weeks this

TABLE 2.—*Sex and Age Incidence of Leiomyosarcoma of the Stomach*

Authors	Sex and Age, Yr.	
	Female	Male
Baumgartner [3]	..	39
Horsley and Berger [4]	62	..
	48	..
	..	34
Chaffin [2]	47	..
	43	..
	17	..
	68	..
	56	..
	65	..
	40	..
	32	..
	32	..
	34	..
	..	39
	..	40
	..	41
	..	33
	21	..
Krauskopf [1]	..	50
Number of each sex	13	7
Average age	43.46	39.42
Age span	17 to 68	33 to 50

burning pain, which at no time radiated, became constant. She was annoyed by large nonbleeding hemorrhoids and by gas in the abdomen. She had lost about 10 pounds (4.5 Kg.) in the past three months "which might have been due to eating only liquids because the pain became worse if solids were eaten." She was nervous and tired all the time. There were no symptoms referable to the head, the neck, the lungs, the extremities, the heart or the urinary system.

Her appetite was good. She was a rapid eater, with proper mastication. There was no difficulty on deglutition. The capacity of the stomach seemed less. There was no belching, heartburn or vomiting. Nausea developed during and after meals. There was hunger-like pain one hour after eating, which may have been due to the fact that the meals ingested were small. The patient never was jaundiced. She was intolerant toward bread and, owing to resultant abdominal pain, was afraid to eat any solid food. The pain of the right lower quadrant of the abdomen did not radiate. There had been some pain intermittently in the left lower quadrant and left loin for the past few months, since hemorroids were noticed protruding on defecation. The bowel movements were regular without medication. The stools were watery; no other gross changes were noted. Recently there had been some pain on defecation.

In her childhood the patient had whooping cough and attacks of sore throat. Twenty-five years prior to examination she had pleurisy with effusion. Twenty-three years before, she underwent appendectomy and uterine suspension.

Her mother died of gastric carcinoma. One brother died of pneumonia. Her father, two brothers and three sisters were living and well. Several paternal relatives were suffering from allergic conditions. There was no history of tuberculosis or of diabetes mellitus.

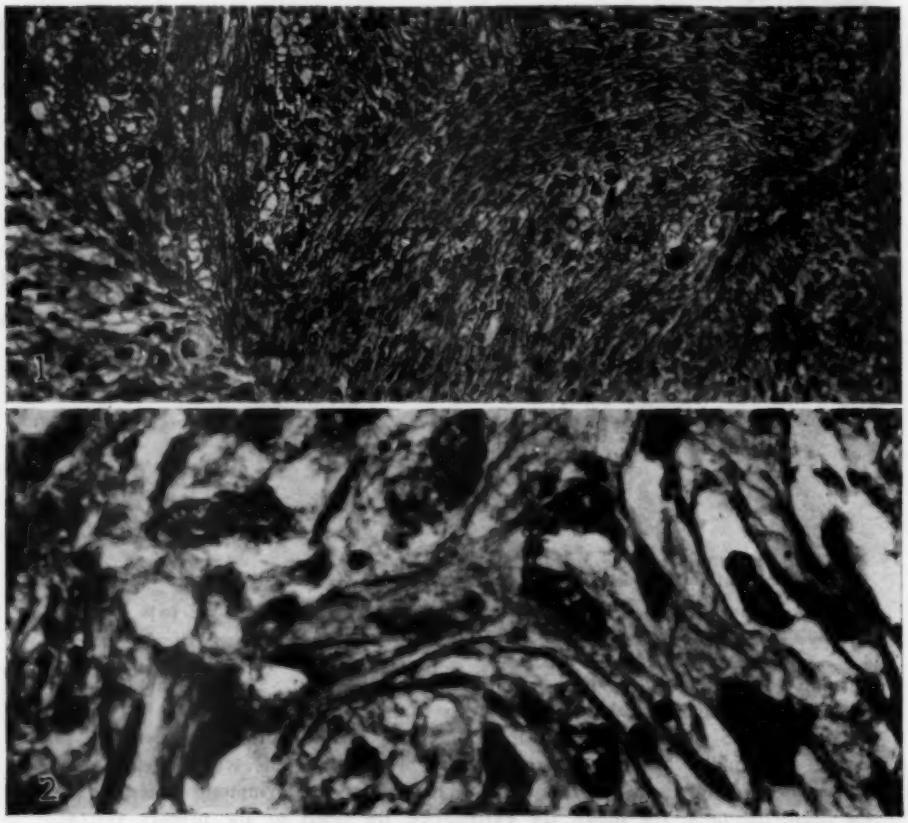

Fig. 1.—Leiomyosarcoma of ureter. Note whorls of tumor cells. These stained yellow by van Gieson's method, as do cells of smooth muscle origin. × 150.

Fig. 2.—Leiomyosarcoma of ureter. Note the giant nuclei of the tumor cells, with bizarre shapes, coarse chromatin particles and large nucleoli. Compare with the nuclei of the slender connective tissue cells, of usual size, interspersed among the tumor cells. × 675.

Examination.—The weight of the patient was 141¼ pounds (64 Kg.). The blood pressure was 140 systolic and 90 diastolic. The pulse rate was 84; the pulse was regular and of good quality. The hemoglobin content was 68 per cent. The reflexes were hyperactive. There were fine tremors of the hands. There was slight prominence of the thyroid gland; no other glands were palpable.

Eyes, ears, nose, throat, teeth and gums presented nothing remarkable. There were no pathologic findings in the breasts. The heart had a presystolic murmur over the apex and a snappy second aortic sound. On fluoroscopy the left auriculoventricular angle was obliterated. In the right lower quadrant of the abdomen there was a mass the size of an orange, movable and tender. There was tenderness over the right upper and the left lower quadrant. Vaginal and rectal examinations presented nothing remarkable.

Cholecystographic study using a double dye method revealed chronic cholecystitis with cholesterol calculi. A flat plate study revealed gaseous distention of the colon with extrinsic pressure on the medial border of the cecum. A gastrointestinal roentgen series revealed duodenal stasis, six hour gastric retention of a small amount of ingested material, with visualization of portions of the small intestine, probably as a result of extrinsic pressure exerted by a mass in the abdomen, a probably retroperitoneal mass in the right lower quadrant. After a barium sulfate enema of the colon there was a shadow suggesting a mass in the right lower quadrant medial to the cecum and the ascending colon. This mass was thought to be retroperitonal. Sigmoidoscopic examination revealed nothing remarkable apart from deduplication of the mucosal folds and internal hemorrhoids. The results of analyses of the stool, the urine and the gastric contents were essentially within normal limits.

The hemoglobin content was 68 per cent (Dare method); the red blood cell count, 4,295,000; the white cell count, 11,800 (neutrophils 53, small lymphocytes 39, large lymphocytes 4, basophils 3 and monocytes 1 per cent). The basal metabolic rate was plus 8.

Operation.—The patient was operated on at the New York Post-Graduate Medical School and Hospital by Dr. Thomas H. Russell, Oct. 23, 1941. A right paracostal incision was made and the abdominal cavity opened. The gallbladder was seen to be tremendously enlarged and edematous. It contained many stones and there was a stone impacted in the cystic duct. However, gallstones were not causing the patient's immediate trouble. A large retroperitoneal tumor was found on the inner side of the ascending colon at a point just to the right of the umbilicus. It was adherent to the peritoneum overlying it. A circular incision was made around the peritoneum so as to remove the diseased peritoneum with the tumor, and the tumor was carefully dissected upward. It was found that the right ureter was densely adherent to the posterior wall of the tumor. The ureter was dissected away from the tumor, and the tumor was removed. The ureter was dissected for a distance of fully 3 inches (7.5 cm.) and, as mentioned, a part of the tumor was left adherent to the ureter. Nephrectomy was considered on account of the adherence of the tumor to the ureter, but on examination the left kidney was found to be a cystic mass, and it was questionable as to how much work the organ was doing. Therefore, removal of the right kidney was out of the question. The liver was examined, and on its inner surface a small nodule the size of the end of the ring finger, looking just like the tumor, was found, which in all probability was metastatic. Frozen section showed the tumor to be a spindle cell sarcoma. The opening in the posterior layer of the peritoneum overlying the tumor was sutured with catgut. Because the patient's symptoms were partly due to obstructive cholecystitis, the gallbladder was opened, and a number of small and large stones were removed. The gallbladder was not removed but was drained by means of a no. 20 rectal tube. A cigaret drain was placed down to the foramen of Winslow, and the drain with the tube was brought out at the lower angle of the wound. The wound was closed with four through

and through silk sutures and in layers with catgut. The skin was closed with silk. The drainage tube was sutured to the skin with silk.

Pathologist's Report.—"Gross Examination: The first specimen consists of 50 faceted yellow friable stones with 3 ounces (88.5 cc.) of golden brown bile. The second specimen is a lobulated mass of pale gray resilient tissue partly covered by a thin capsule with attached yellow lobules of fat and measuring 9 by 5 by 4.5 cm. On section there is pale yellowish gray tissue, which is finely striated to form ill defined whorls. There are also some pale gray translucent areas several millimeters in diameter scattered at various points.

"Microscopic Examination: Paraffin-embedded sections show the following: Wide bands of spindle-shaped cells form an intertwining pattern with solid wide sheets of cells, and also weave a convoluted course through areas of necrosis. The cells vary in size, shape and staining properties. Simple spindle shapes are intermingled with thick short types, the latter often with giant and bizarre nuclei. Some are multinucleated, and most have rounded granular nuclei, varying greatly in size, and prominent nucleoli. Mitotic figures and bizarre and multinucleated forms are occasionally seen. With appropriate methods of staining the neoplastic spindle cells show myofibrils, by which they are identified as smooth muscle cells.

"Diagnosis: Gallstones; leiomyosarcoma."

Postoperative Course.—The patient made an uneventful recovery from the operation. After an asymptomatic period of about three months there was a return of the pain in the right lower quadrant of the abdomen. The course thereafter was progressively downhill, and about two years after operation the patient died from generalized metastases.

SUMMARY

A leiomyosarcoma of the right ureter has been observed. No other cases of leiomyosarcoma of this site could be found recorded.

A tabulation of unselected cases of leiomyosarcoma reveals that the frequently repeated statement that sarcoma occurs principally in the young and carcinoma in the middle-aged and the aged is not valid so far as leiomyosarcoma is concerned.

The ratio of female to male patients is approximately 2:1 when one does not consider the incidence of uterine leiomyosarcoma. With that incidence included, the ratio would be closer to 3:1.

PARATHYROID ADENOMA, WITH UREMIA DUE TO CALCIFICATION OF THE KIDNEYS

PAUL LOBER, M.D.; AMBROSE J. HERTZOG, M.D., and CARL O. RICE, M.D.
MINNEAPOLIS

IT IS well known that primary hyperparathyroidism promotes the formation of renal calculi, but it is not so widely appreciated that it may injure the renal parenchyma and cause renal insufficiency. Cases of primary hyperparathyroidism associated with marked renal insufficiency have been reported by Elsom, Wood and Ravdin,[1] Baker and Howard [2] Bellin and Gershwin,[3] Albright, Baird, Cope and Bloomberg [4] and others. In some cases, such as that reported by Curtis and Feller,[5] it is difficult even at autopsy to determine whether the renal insufficiency preceded or followed the hyperparathyroidism. A review of the whole subject has been contributed by Anderson.[6]

The case reported here is of special interest because it was observed postoperatively for three years before death occurred from uremia. After the surgical removal of the parathyroid adenoma, renal insufficiency persisted from severe calcification of the kidneys. A state of secondary or compensatory hyperparathyroidism occurred as a result of the lowering of the serum calcium and the elevation of the serum phosphorus. It closely resembles the case reported by Downs and Scott [7] and the third case of Soffer and Cohn.[8] It illustrates many of the changes that can occur in calcium and phosphorus metabolism, bones, kidneys and parathyroid glands as a result of disturbances of parathyroid and renal function.

REPORT OF A CASE

A woman 43 years of age was first admitted to St. Barnabas Hospital July 6, 1940. She was single, working as an office clerk, and had been in fair health until 1933. Her illness apparently began in 1933 with progressive nervousness. She later became confused and was unable to think accurately or concentrate. She was forced to quit work. She complained of weakness and cardiac palpitation. At this time examination revealed nothing of note except a blood pressure of 148-systolic and 104 diastolic with a pulse rate of 106 per minute and evidence

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1. Elsom, K. A.; Wood, F. C., and Ravdin, S.: Am. J. M. Sc. **191**:49, 1936.
2. Baker, B. M., Jr., and Howard, J. E.: Bull. Johns Hopkins Hosp. **59**: 251, 1936.
3. Bellin, D. E., and Gershwin, B. S.: Am. J. M. Sc. **190**:519, 1935.
4. Albright, F.; Baird, P. C.; Cope, O., and Bloomberg, E.: Am. J. M. Sc. **187**:49, 1934.
5. Curtis, L. E., and Feller, A. E.: Ann. Int. Med. **17**:1005, 1942.
6. Anderson, W. A. D.: Arch. Path. **27**:753, 1939.
7. Downs, R. S., and Scott, V.: Arch. Int. Med. **67**:658, 1941.
8. Soffer, L. J., and Cohn, C.: Arch. Int. Med. **71**:630, 1943.

of recent loss of weight. The urine showed a specific gravity of 1.016, a faint trace of albumin and nothing of significance on microscopic examination. She was discharged, August 21, with a diagnosis of psychoneurosis. Following this, her symptoms persisted in greater or lesser degree and were associated with numerous other vague complaints. In November of 1941 she began to complain of arthritic pain. She stated that all of her joints were stiff and painful. In May of 1942 she became sensitive to heat and cold. At about this time she noticed some deformity of the bones of her forearms and also increasing fatigue and weakness. She stated that her hats had become too small for her during the past year. She had experienced nocturia and diurnal frequency of urination for about one year. Recently her vision had been blurred, and she complained of a frequent dull headache. She reentered St. Barnabas Hospital on Aug. 18, 1942.

She was acutely ill and extremely emaciated. The frontal bones of the skull were prominent. The eyes, the ears, the nose and the throat were normal. The thyroid gland was slightly enlarged but was symmetric and of normal consistency. At the right lower pole was a small, barely palpable mass giving the impression of an adenoma of the lower pole of the thyroid gland. A definite systolic and diastolic bruit could be heard over the thyroid gland. The chest and the lungs were normal. The pulse rate was 120 per minute at rest, and the blood pressure was 140 systolic and 92 diastolic. The heart seemed slightly enlarged on percussion. The abdomen was pendulous, and the abdominal muscles lacked tonicity. The liver, the kidneys and the spleen could not be palpated. There was a noticeable bilateral bowing of the forearms. The long bones were sensitive to pressure. There was an atrophic type of dermatitis over both feet. The reflexes were equal and hyperactive. Laboratory examination revealed a hemoglobin content of 76 per cent (Sahli), with 9,650 leukocytes. A differential count showed 65 per cent neutrophils. The smear showed mild hypochromasia of the red blood cells. The urine had a specific gravity of 1.012, with a trace of albumin; microscopic examination revealed no abnormity. Bence Jones protein was absent. A Sulkowitch test for urinary calcium was positive with a 1 to 2 plus reaction. Serum calcium was 16.6 mg., and serum phosphorus was 6.8 mg., per hundred cubic centimeters; alkaline phosphatase was 52.3 King-Armstrong units. Total plasma protein was 5.5 Gm. per hundred cubic centimeters. Blood urea nitrogen was 51.8 mg. A recheck of the serum calcium showed a calcium level of 16.4 mg. and a phosphorus level of 6.15 mg. Roentgenologic examinations of the skull, the pelvis, the lumbar spine and the long bones were made. There was marked thickening of the bones of the skull, with areas of increased density and rarefaction. The changes in the skull suggested Paget's type of osteitis deformans. The pelvis, the left tibia, the right ulna and the radius, the left clavicle and the upper end of the left femur contained multiple cysts as found in generalized osteitis fibrosa cystica. August 22 a biopsy specimen was taken from a cystic lesion of the left clavicle. The biopsy showed osteitis fibrosa cystica. Two days later this clavicle fractured spontaneously. The clinical diagnosis was generalized osteitis fibrosa cystica due to a parathyroid adenoma.

August 25 the patient was operated on by one of us (C. O. R.).[9] The thyroid gland appeared entirely normal. Posterior to the right lower pole was a brownish red tumor measuring 3.5 by 2 by 1.5 cm. and weighing 3.17 Gm. (fig. 1). It was well encapsulated and extended into a recess in the posterior wall of the thyroid gland. The tumor was removed intact. Histologic examination showed

9. Rice, C. O.: Minnesota Med. **26**:1092, 1943.

it to be a parathyroid adenoma composed largely of chief cells, with scattered areas of the vesicular cells present (fig. 2). Postoperatively the patient almost immediately presented signs of tetany. She became restless and nervous and later had several emeses. On the day after the operation the serum calcium dropped to 7.3 mg. per hundred cubic centimeters. The serum phosphorus remained elevated at 5.4 mg. Four days after the operation the serum calcium was 6.3 mg. The Sulkowitch test for urinary calcium became negative after the operation. The postoperative hypoparathyroidism was controlled by giving 1 cc. of parathyroid injection U. S. P. parenterally on two days. Following this, she received dihydrotachysterol. She was discharged from the hospital September 6 and was advised to take calcium, vitamin D and dihydrotachysterol. She felt greatly improved and had gained in weight and strength.

May 11, 1943 roentgenograms were made of the legs, the thighs, the clavicles, the skull and the right forearm. These showed an improvement in the cystic lesions involving the various bones. The lesions had not completely disappeared. For nearly two years after the operation the patient felt well. She continued to take calcium by mouth and vitamin D. In her third postoperative year she complained of nocturia and excessive thirst. She later became weak and suffered from severe nausea and vomiting. She was admitted to the Minneapolis General Hospital on Aug. 22, 1945. Physical examination revealed an apathetic, emaciated woman 48 years of age. Her blood pressure was 90 systolic and 70 diastolic. The skin had a dark color and was very dry. The temperature, the respirations and the pulse were normal. There were scattered areas of ecchymosis measuring up to 2 cm. in diameter over the trunk and the extremities. The heart and the lungs revealed no abnormalities. The abdominal regions appeared normal. The extremities were extremely tender on pressure. Chvostek's sign was questionably positive. The patient was somewhat confused and disoriented and complained of multiple pains. The hemoglobin content was 72 per cent (Sahli). Serologic tests revealed no syphilis. The leukocyte count was 10,400, with 81 per cent neutrophils. The urine had a specific gravity of 1.015, contained albumin (3 plus) but no sugar and showed one to two red blood cells per high power microscopic field. Blood sugar was 128 mg. per hundred cubic centimeters; the carbon dioxide-combining power was 47 volumes per cent; the serum calcium was 8.77 mg. per hundred cubic centimeters and when rechecked was found to be 6.4 mg. Serum phosphorus was 10.2 mg. Alkaline phosphatase was 10.4 King-Armstrong units. Serum albumin was 6.4 Gm. and serum globulin was 3.0 Gm. per hundred cubic centimeters. Blood urea nitrogen was 174 mg., with 6.3 mg. of creatinine. Roentgenograms of the skull and long bones showed changes that were consistent with partially healed generalized osteitis fibrosa cystica. In some areas the process appeared to be still active suggesting persistent hyperparathyroidism. Punched-out areas were present in the skull. Aspirated sternal marrow revealed no evidence of any blood dyscrasia, multiple myeloma or metastatic carcinoma. A second determination of carbon dioxide-combining power showed it to be 19 volumes per cent. The clinical diagnosis was uremia. She died on Aug. 28, 1945.

Postmortem Examination.—The body was that of a fairly well developed but poorly nourished white woman of middle age. There was no jaundice or edema. There were scattered areas of ecchymosis over the surfaces of the legs and the arms. The peritoneal cavity contained about 200 cc. of amber fluid. The surfaces were smooth and glistering. The right pleural cavity contained about 500 cc. of straw-colored fluid and the left 300 cc. of similar fluid. The pericardial sac appeared normal. The heart weighed 250 Gm. and appeared normal. The right

lung weighed 260 Gm. and the left 410 Gm. Both lungs showed slight edema and small areas of hypostatic pneumonia. The liver weighed 1,320 Gm. and was not remarkable. The bile ducts were patent, and the gallbladder appeared normal. The spleen weighed 100 Gm. The gastrointestinal tract appeared normal. The right kidney weighed 90 Gm. and the left 110 Gm. The capsules stripped with ease from surfaces that were granular and deeply scarred. The right kidney showed considerable atrophy of the parenchyma, as the cortex and medulla in

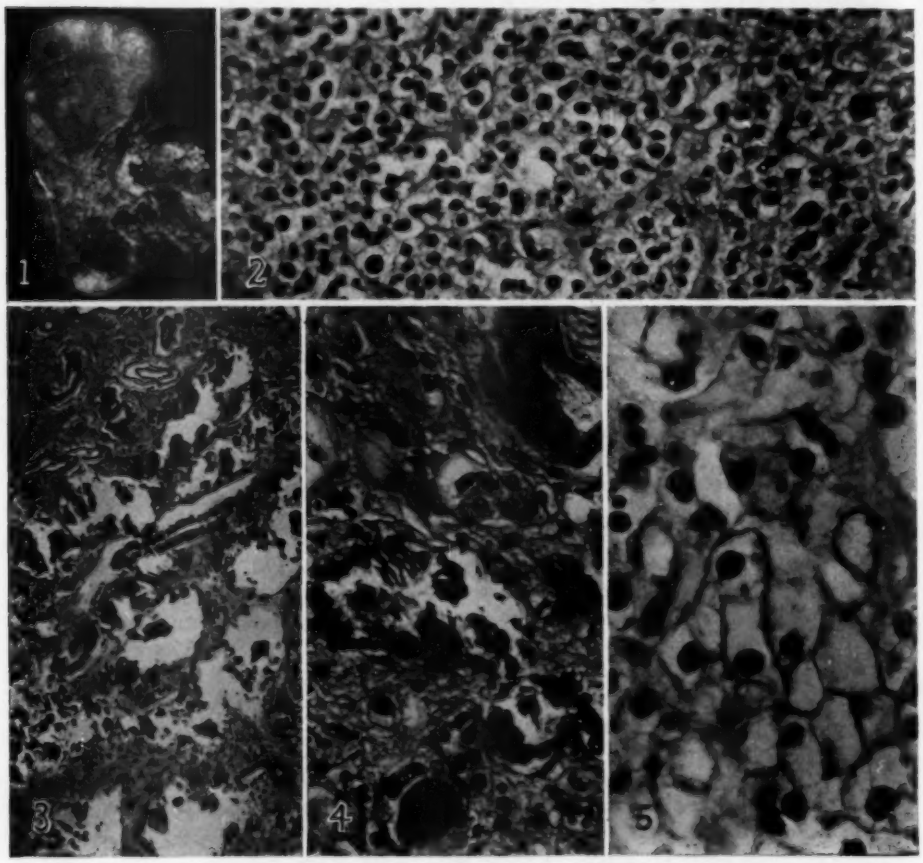

Fig. 1.—Parathyroid adenoma.

Fig. 2.—Photomicrograph of the parathyroid adenoma.

Fig. 3.—Photomicrograph of kidney showing cystic spaces filled with calcium.

Fig. 4.—Photomicrograph of kidney showing calcium deposited in tubules.

Fig. 5.—Photomicrograph of parathyroid gland at autopsy showing preponderance of "wasserhelle" cells.

some areas measured only 3 mm. in thickness. The cortex of the left kidney measured 3 mm. and the medulla 10 mm. at the widest point. The markings between the cortex and the medulla were indistinct in both kidneys. The right kidney contained, in addition, a number of small cortical cysts measuring up to

2 cm. in diameter. Stones or gross evidence of calcification were not seen in either kidney. The pelvis of the right kidney showed slight dilatation. The left pelvis appeared normal. The ureters and the bladder appeared normal. The genital organs revealed nothing of note. The aorta showed moderate sclerosis. There was no enlargement of the thyroid gland. Two parathyroid glands were found in the connective tissue behind the left lobe of the thyroid. The remaining parathyroid gland of the right side could not be found. The two parathyroid glands found measured 5 by 3 by 2 mm. each. The ribs and the sternum showed no gross abnormalities. The skull, the long bones, the brain and the spinal cord were not examined.

Microscopic Examination.—Kidneys: Under low magnification the most impressive alteration was the presence of calcium deposits throughout the parenchyma (fig. 3). The calcium was much more abundant in the medullary pyramids than in the cortex. In the pyramids there were many cystic spaces filled with calcium, which were almost of macroscopic dimensions. There was also rather marked patchy atrophy of the cortical tubules, and some of the intact tubules were moderately dilated. Under high magnification it appeared that the cystic spaces in the pyramids represented collecting tubules distended with calcium. Since the lining epithelial cells had disappeared at the site of the calcium deposit, it was not easy to determine whether the calcium was intratubular or interstitial. Nearby all the collecting tubules were destroyed. A careful study revealed that the calcium in the cortex was intratubular (fig. 4) although the destruction of the tubular epithelium often caused it to appear as a deposit in the interstitial tissue. There was a moderate lymphocytic exudate throughout the atrophic areas. In addition to the obstructive tubular lesion, which was the cause of the uremia, there were a number of hyaline glomeruli associated with atrophic tubules. Some of the hyaline glomeruli were surrounded by old fibrous crescents. The vast majority of the glomeruli were normal.

Parathyroid Glands: Sections of the two remaining glands found at autopsy showed a marked preponderance of large clear *wasserhelle* cells, with a few oxyphil and chief cells present (fig. 5).

Remaining Organs: The sections showed nothing of note other than an inflammatory exudate in the lungs as found in the terminal stage of bronchopneumonia.

COMMENT

The patient's condition can be classified as primary hyperparathyroidism due to an adenoma of one parathyroid gland. The symptoms related to the skeletal system began about nine years before operation. There was no history of any preceding renal disease. The changes in the blood chemistry were the result of hyperparathyroidism as modified by secondary renal insufficiency. The serum calcium levels of 16.6 mg. and 16.4 mg. were obtained before operation at the time the serum phosphorus was 6.8 mg. This did not fit the usual picture of hyperparathyroidism, as one expects a reciprocal relationship of low phosphorus with high serum calcium. The answer was found in the blood urea nitrogen level of 52.8 mg. This indicates that at the time of the operation there was a considerable degree of renal insufficiency with loss of the ability to excrete phosphates. The serum calcium, however, remained high under the influence of the parathyroid adenoma. Following operation, the serum calcium fell to the tetanic levels of 7.3 mg. and 6.3 mg. This is positive evidence that the hyperparathyroidism was due to the adenoma and not to diffuse hyperplasia of all four

glands. The serum phosphorus remained elevated at 5.4 mg. This elevation of serum phosphorus may have been another factor in the depression of the calcium level after the stimulus of the parathyroid adenoma had been removed. Unfortunately, further studies of both calcium and phosphorus metabolism were not done until the patient's final admission to the Minneapolis General Hospital three years later. At this time her serum calcium varied between 6.4 mg. and 8.7 mg., while the serum phosphorus was 10.2 mg. The histologic discovery of the *wasserhelle* cells in the remaining parathyroid glands suggests a compensatory hyperfunctioning state to compensate for the depression of serum calcium levels. The findings in the long bones and the skull shortly before death suggested both a healing of the initial lesions associated with osteitis fibrosa cystica and further progression of the disease within the bones. The serum phosphorus rose from 5.4 mg. to 102 mg. during this three year postoperative period. The blood urea nitrogen rose from 51.8 mg. to 174 mg. during the same period. The reciprocal relationship between phosphorus and calcium levels placed a severe strain on the calcium metabolism postoperatively and led to a state of secondary hyperparathyroidism.

The renal destruction as a result of the deposition of calcium was extensive. The tubular blocking appeared to be of long standing and to have arisen in the years prior to her operation. Dr. E. T. Bell studied the kidneys in this case for us. The old fibrous crescents about the hyaline glomeruli indicated that the patient at one time had glomerulonephritis and that a number of glomeruli had been destroyed. The hyalinization of a small percentage of the glomeruli was not the cause of the renal insufficiency. This was clearly due to the widespread presence of calcium casts obstructing the tubules, notably the collecting tubules. The advisability of long-continued postoperative oral administration of calcium and vitamin D has to be questioned because of the danger of further calcification of the kidneys, already damaged. Tumulty and Howard [10] showed that excessive intake of calcium with massive vitamin D therapy may cause prolonged hypercalcemia and persistent impairment of renal function in normal persons. The fact that this patient responded well to the surgical removal of the adenoma, with a state of fair health resulting over a period of two years, would justify the removal of a parathyroid adenoma even in the presence of considerable renal damage. The prognosis for complete cure seems poor.

The parathyroid adenoma consisted largely of chief cells, with scattered areas of large vesicular cells. The *wasserhelle* cells were absent. On the other hand, the parathyroid glands removed at autopsy were composed almost entirely of *wasserhelle* cells. The latter picture has been described by Castleman and Mallory [11] as typical of diffuse primary hyperplasia, but in this case all evidence points to the change being secondary to the renal insufficiency. Nelson [12] found no constant uniformity in the histologic aspects of the parathyroid glands in cases

10. Tumulty, P. A., and Howard, J. E.: J. A. M. A. **119**:233, 1942.

11. Castleman, B., and Mallory, T. B.: Am. J. Path. **13**:553, 1937.

12. Nelson, A. A.: Arch. Path. **24**:30, 1937.

of renal insufficiency, as in some cases reported the glands were composed mainly of chief cells; in others, of *wasserhelle* cells, and in others, of various mixtures and transitional forms.

SUMMARY

Primary hyperparathyroidism due to a parathyroid adenoma caused uremia in a 45 year old woman. Death occurred three years after the surgical removal of the parathyroidoma. Renal insufficiency was present at the time of the operation, with subsequent elevations of the blood levels of phosphorus and urea nitrogen. At autopsy uremia was found to be due to calcification of the tubules of the kidneys. The roentgenograms of the bones and the appearance of the remaining parathyroid glands when encountered at autopsy suggested a postoperative state of gradually increasing secondary hyperparathyroidism due to the progressive renal failure.

SPLENIC HEMANGIOSARCOMA

A Case with Lymphatic and Vascular Metastases

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AT LEAST 9 cases of primary splenic hemangiosarcoma have been reported, as outlined in tables 1 and 2. This rare tumor has usually been recognized on microscopic examination after splenectomy or autopsy. In most of the reported cases the spleen was three to ten times normal size. Symptoms (and sometimes death) were related to splenomegaly and hemorrhage from the primary site. With the exception of several cases in which curative splenectomy was accomplished before metastases occurred, secondary growths in the liver were common. Metastases elsewhere have not been commonly noted, and metastases in lymph nodes have not been previously reported.

REPORT OF CASE

A 32 year old white married woman bore a full term, healthy child in the summer of 1942. For relief of repeated sore throats, tonsillectomy had been performed in the seventh month of her pregnancy. About that time she complained of weakness, which persisted until her death. In May 1943 pain began in the right side of the chest, accentuated by deep respiration. Following delivery, her sore throats had recurred; therefore in June 1943 some tonsillar tags were removed. In July she complained of a sensation of fulness in the upper part of the abdomen and shortness of breath on exertion. A nonproductive cough was noted. She was afebrile and was not losing weight. In August she entered the hospital, complaining of "pleurisy." Enlargement of the posterior cervical lymph nodes was noted. The node at the angle of the right mandible was large, fixed and not tender. During her stay in the hospital it was excised and examined (a report is given later in this article). Bilateral pleuritic pain was experienced on deep inspiration; expansion was poor, and voice and breath sounds were slightly altered. Tenderness was noted at the right costal margin. The hemoglobin content was 84 per cent; the erythrocyte count, 4,500,000, the leukocyte count, 9,600. The urine was normal. The basal metabolic rate was minus 7. The sputum when examined showed nothing remarkable. Dextrose tolerance tests gave results within normal limits. The sedimentation rate was persistently elevated. A roentgenogram of the chest showed soft, small, mottled shadows in the lower lung fields, increased linear markings and increased density about the right hilus. Roentgenograms taken at intervals of several weeks showed continued increase in the density of the linear markings. A lateral roentgenogram of the skull showed one clear, fairly smooth, rounded area of decreased density in the right occipital region, probably in the outer table. In the roentgenograms of the spine, areas of decreased density were seen in the second and third lumbar bodies.

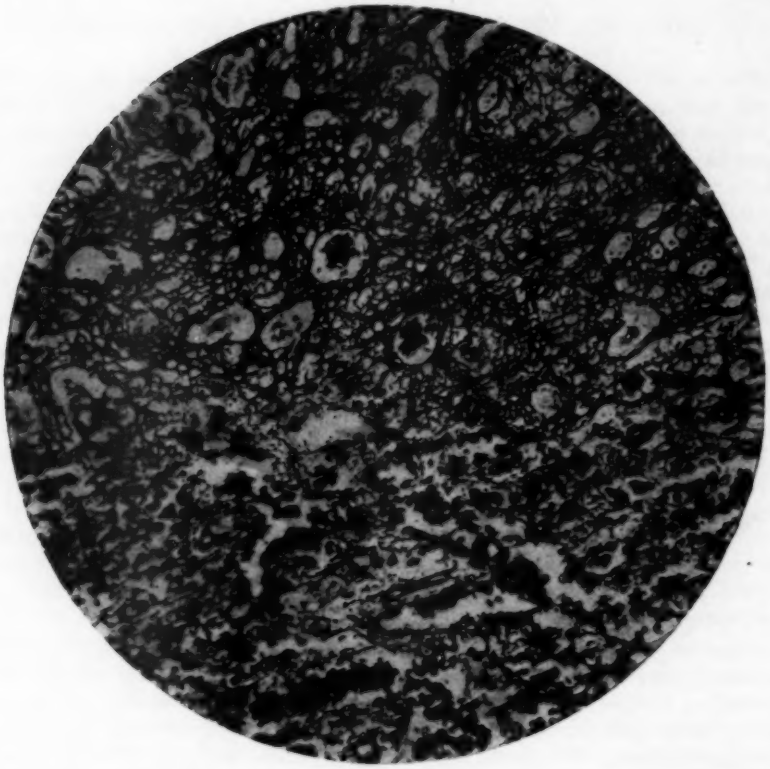
From the Department of Pathology, Duke University School of Medicine and Duke Hospital, and the Department of Medicine, Watts Hospital.

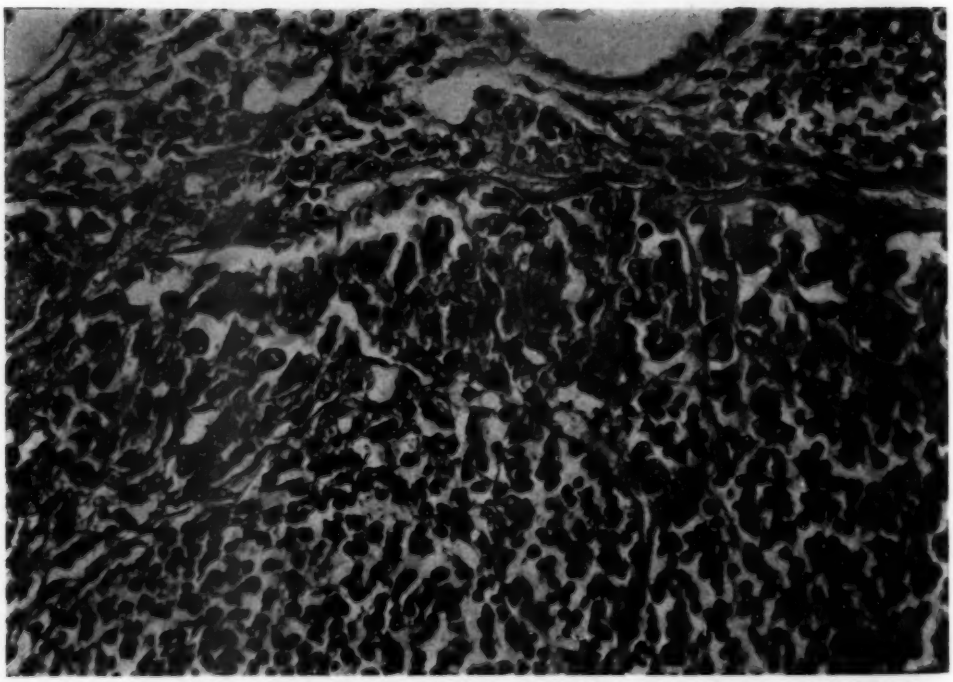
The patient complained often of pain in the lumbar region and the lower part of the right hemithorax. Her throat was "sore" much of the time, but nothing to explain this was found. Biopsy of the right submandibular node indicated the probability of a cancer metastasizing to the cervical lymph nodes, primary site unknown. The recognition of an "atypical growth of polyhedral cells with large nuclei and indefinite cell outlines, the cells looking as though of epithelial origin," and the finding of infrequent mitotic figures, left small room for question whether the illness was infectious or neoplastic. The patient left the hospital in September 1943.

Her course during the month's interval before the terminal admission was characterized by a gradual onset of hemoptysis, increased weakness and slight pyrexia. On admission she was acutely ill, very orthopneic, dyspneic and cyanotic. Her temperature was 39.8 C. (103.6 F.); the pulse rate, 140; the blood pressure, 100 systolic and 80 diastolic. She was still adequately nourished. Submandibular and submaxillary nodes were slightly enlarged and not tender. The chest was dull to percussion in the axillas and the bases of the lungs. Coarse, sticky rales were heard. The abdomen was distended, with the liver palpable 3 fingerbreadths below the costal margin. The hemoglobin content was 75 per cent; the erythrocyte count, 3,900,000; the leukocyte count, 17,900. The patient became rapidly worse despite oxygen therapy, dying sixteen hours after admission, one and one-half years after the onset of the illness.

Autopsy.—The spleen was average size (145 Gm.). Externally there was nothing to suggest the presence of the spherical tumor 2.5 cm. in diameter discovered on section. The remainder of the spleen was entirely normal in consistency, color and structure except for a few light yellow tubercles immediately beneath the capsule. The tumor was a single circumscribed round nodule, bulging from the cut surface. It was mottled purple and dark gray, and therefore presented little contrast to the surrounding normal splenic tissue. Several pancreaticolienal, hepatic and periaortic lymph nodes were slightly enlarged. On section these were mottled with gray-purple areas. The 1,570 Gm. liver had many capsular-diaphragmatic adhesions. In such places there were gumma-like lesions with gray-purple color, necrotic-looking centers and multiple pseudopod-like peripheral extensions into the hepatic parenchyma. Most of these were 1 to 3 cm. in diameter. Within the depths of the organ were similar areas, some of which were strikingly hemorrhagic. The right pleural cavity contained 2,000 cc. of sanguineous fluid without gross blood clot. A moderate quantity of fibrin was found on the right pleural surfaces, and one basilar adhesion. There was neither adhesion nor effusion on the left. A marked contrast was noted bilaterally between the purple color of most of the visceral pleural surface and the white color of the broad linear trabecular markings. The lungs were noncrepitant and extraordinarily firm, as though completely solidified. A decidedly hemorrhagic tissue was revealed by section, but diffusely scattered areas of air-containing lung substance persisted. Together the lungs weighed 1,560 Gm. The pigmented hilar nodes were not enlarged. Hemorrhagic, spongy areas were encountered in the bodies of the twelfth thoracic and second and third lumbar vertebrae. These averaged 2 cm. in diameter. The 250 Gm. heart was dilated in all chambers. The right side was more dilated than the left. Permission was refused for examination of the brain and the area of decreased radiodensity in the skull which was reported in the clinical studies.

Microscopic Examination.—Spleen: Sections from outside the grossly visible tumor area showed no lesions except several fibrocaseous tubercles immediately





Upper: The characteristic reticulum of the tumor (lower part) is shown in contrast with the normal reticulum of the adjacent normal splenic tissue (× 110).

Lower: Microscopic appearance of a solid tumor metastasis in the peripheral sinus of a pancreaticolienal lymph node (× 341).

beneath the capsule. The circumscribed area of tumor was not encapsulated. The transition between normal splenic and neoplastic tissue was gradual, being evidenced most obviously by increased vascularity. There was, however, an abrupt change in the reticulum, as revealed by the Perdrau stain. In the tumor the reticulum was denser. Mallory's connective tissue stain left no doubt that there was an increase in collagenous tissue. The principal cellular component of the tumor was a pleomorphic cell. The cells lined the irregular blood-filled channels, often forming a stratified layer two or three cells thick. Tumor-containing thrombi and tumor cells without thrombi were found within normal splenic blood vessels. In some places the tumor cells simulated fibroblasts; elsewhere, macrophages; in still other places, endothelial cells lining the normal splenic sinuses. They were poor in cytoplasm, but protoplasmic processes, apparently connecting with the reticulum, were discernible in places. The clear ovoid nuclei contained granules and threads of basophilic material, principally at the periphery. They varied between 10 and 20 microns. Nucleoli were not found. Mitoses and multinucleated cells were rare. Zones of necrosis were not found.

Tumor in Other Sites: The features described for the splenic nodule were characteristic of the metastases found in abdominal lymph nodes, the liver, the lungs, the mediastinal lymph nodes and the vertebrae. Necrotic changes were most pronounced in the liver. Because there was a moderate grade of central necrosis secondary to passive congestion outside the tumor areas in the liver, it seems improper to ascribe much weight to the degree of necrosis within the hepatic tumor in comparing the neoplastic lesions in the spleen and the liver as to age. The tumor growth in the pleura, the pleural lymphatics and the interlobular septums was extensive. Hemorrhage was widespread, completely filling many alveoli and bronchioles. The tumor stroma was densest in the vertebral site, where relatively avascular hyalinized regions were observed.

Other Tissues: Sections of the thymus, the submaxillary gland, the thyroid gland, the breast, the pancreas, the adrenal glands, the kidneys, the ileum, the heart and the ribs showed no tumor. No trace of tonsillar tissue remained for microscopic study.

COMMENT

Two tables are included in this report for the purpose of demonstrating the similarities and the differences between this and the previously reported cases of cancerous primary splenic hemangioma.

The extraordinary papillary projections noted by Wright [1] were not present in this case. Apparently the abnormal vascular channels elaborated by hemangiosarcoma are connected with the normal blood vessels and function, however inadequately, as a blood supply for the tumor cells which line them. In cases in which necrosis of tumor was noted in the spleen, thrombi and infarcts were prominent.

The small size of the tumor in the spleen observed by us might be considered as an argument against the conclusion that the spleen was the primary site. The following were considered as possible sites of the primary tumor: (1) tonsils; (2) bones, especially the vertebral bodies; (3) liver; (4) spleen. The conclusion that the spleen was the primary site is based on the character of the tumor itself and the general character and distribution of the metastases. In the spleen

1. Wright, A. W.: Am. J. Path. 4:507, 1928.

the tumor was a single circumscribed nodule. Everywhere else it was multiple and infiltrating. The characteristic microscopic structure was nowhere as definitive as in the spleen. It may be significant that intravascular growth was venous in the spleen and primarily portal in the liver. Lymphatic spread from the spleen was suggested by the involvement of the pancreaticolienal and hepatic lymph nodes. In most reported cases the spleen was known to be involved in some sort of

TABLE 1.—*Gross Features of Reported Cases*

Author	Date	Wt. of Spleen, Gm.	Age	Race	Sex	Metastases
DeNavasquez, S.: J. Path. & Bact. **42**: 651, 1936	1936	525	57	White	M	Liver
Garlock, J. H.: J. Mt. Sinai Hosp. **6**: 319, 1940	1940	635	13	White	F	Splenectomy; recovery
Homans, J.: Ann. Surg. **25**: 732, 1897	1897	415	22	White	F	No autopsy
Jopson: S. Clin. North America **2**: 235, 1921	1921	1,500	33	White	F	Splenectomy; recovery
Von Jores, L.: Zentralbl. f. allg. Path. u. Path. Anat. **19**: 662, 1908	1908	3,600	45	White	F	Liver
Langhans, T.: Virchows Arch. f. path. Anat. **75**: 273, 1879	1879	2,880	30	White	M	Liver
Paine, C. G.: J. Path. & Bact. **34**: 139, 1931	1931	1,300	64	White	M	Liver and bone
Thelle: Virchows Arch. f. path. Anat.	1904	2,500	56	White	M	Liver, lungs and stomach
Wright [1]	1928	520	25	White	M	Liver
Bauer, D. deF., and Stanford, W. R.: Arch. Path., this issue	1944	145	32	White	F	Liver, pleura, lungs, bronchial, cervical and retroperitoneal lymph nodes and vertebrae

TABLE 2.—*Microscopic Features of Reported Cases*

Author *	Cell Type	Giant Cells	Mitoses	Solid Growth	Degenerative Changes
DeNavasquez.....	Spindle	Present	Numerous	Present	Liver only
Homans.....	.....	.....	.....	.....	Hyalinization
Jopson.....	Spindle	Present	.....	Present	Hyalinization
Jores.....	.....	.....	.....	.....	Marked necrosis
Langhans.....	Fusiform	.....	.....	.....	.....
Paine.....	Spindle to circular	Present	Rare	Present	Necrosis
Thelle.....	Endothelial	.....	.....	Present	.....
Wright.....	Large vesicular	.....	Numerous	Present	Necrosis
Bauer and Stanford	Polyhedral	Present	Rare	Present	Necrosis

* For complete references, see table 1.

disease process because of its large size. Here the small size of the primary splenic tumor removed the possibility of diagnosing the ailment from localizing symptoms.

An additional factor which increased the difficulty of diagnosis was the surprisingly extensive growth of the tumor in the lungs. Although it soon became apparent that the process was neoplastic rather than infectious, interest continued to center in the progressive pulmonary

involvement because the most disturbing of the symptoms and the cause of death could be traced to this. Increasing obstruction of pulmonary vessels by embolism and the pressure of tumor growth resulted in progressively rising pulmonary blood pressure. The right ventricle was at last incapable of further compensation. The massive right pleural effusion was one evidence of the decompensation which ensued. Concomitant atelectasis from pressure of this effusion on the small amount of remaining lung tissue of the right side further increased the cardiac burden. When the load was then no longer bearable, cardiac dilatation occurred with general circulatory failure and death.

SUMMARY

Of the 7 reported cases of splenic hemangiosarcoma in which autopsy was performed, the case presented here is the only one in which metastases were demonstrated in lymphatic and lymphoid structures. This case is likewise interesting because of the small size of the primary tumor in relation to the large size and the wide distribution of the metastases. The wide distribution of intrapulmonary metastases produced pulmonary hypertension and myocardial failure.

Laboratory Methods and Technical Notes

A RAPID GRAM STAIN FOR TISSUE

EDWIN M. LERNER II, M.D., BOSTON

A NEW modification of the Gram stain for the demonstration of bacteria in tissues has been employed in this laboratory with success. The principal objections to the usual modifications of the Gram stain previously employed are: the length of time required, the numerous and sometimes complicated steps in the procedures and the fact that many of the Gram stains on tissue give little or no differentiation between gram-positive and gram-negative organisms. Furthermore, the commonly employed Gram-Weigert modification gives poor delineation of both bacterial and tissue detail.

PROCEDURE

The procedure is essentially that of the Gram stain commonly employed for bacterial smears. In point of fact, the staining may be done on individual slides in a fashion identical with that used for staining bacterial smears. It is more convenient, however, when staining several slides at once to use staining jars.

PREPARATION OF SLIDES

Fix tissue in Zenker's solution twenty-four hours.
Embed in paraffin.
Cut sections 6 microns thick.
Mount on slides and heat at 56 C. two to six hours.

STAINING SOLUTIONS

Stirling's Gentian Violet Solution

Crystal violet	5 Gm.
Absolute alcohol	10 cc.
Aniline	2 cc.
Distilled water	88 cc.

Iodine Solution

Iodine	1 Gm.
Potassium iodide	2 Gm.
Distilled water	100 cc.

Decolorizing Agent

Acetone	50 cc.
Absolute alcohol	50 cc.

Safranin

1 per cent aqueous solution

From the Department of Pathology, Children's Hospital and Infants' Hospital.

METHOD OF STAINING

1. Treat paraffin sections in xylene ten minutes, then in absolute alcohol ten minutes; rinse in 95 per cent alcohol.
2. Stain with Stirling's gentian violet one minute.
3. Wash in water.
4. Treat with iodine solution one minute.
5. Wash in water.
6. Decolorize with alcohol-acetone mixture until no more stain comes away (ten to thirty seconds).
7. Wash in water.
8. Counterstain with safranin thirty seconds.
9. Wash in water.
10. Rinse in 95 per cent alcohol until excess safranin is removed.
11. Rinse in absolute alcohol, then in xylene, and mount in balsam.

RESULTS

Gram-positive organisms are stained deep blue to violet; gram-negative organisms, red. The nuclei of cells are stained pink to red, with excellent intranuclear detail, and the cytoplasm is stained light yellowish pink. Bacterial spores are easily visible as clear spaces within the cells.

COMMENT

The rapid Gram stain for differential staining of bacteria in tissues requires less than five minutes of actual staining time for its execution and has the added advantage of being extremely simple. It gives excellent morphologic detail of both bacteria and tissues, although the latter are stained somewhat lightly. Spore-bearing bacteria can be recognized readily. Bacteria are sharply delineated whether occurring in numbers or scattered individually through the tissues. The finding of occasional scattered organisms is thus facilitated.

A HEMOGLOBIN STAIN FOR HISTOLOGIC USE BASED ON THE CYANOL-HEMOGLOBIN REACTION

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THE USE of cyanol as a stain for hemoglobin was suggested by Fautrez and Lambert,[1] whose technic required one to twenty-four hours. In addition, the preparation of the staining solutions called for precisely made buffers and boiling of carmalum for one hour. This elaborate preparation was undoubtedly necessary in staining the delicate hemoglobin globules in the renal epithelium of Urodeles, as they did, but with larger laboratory animals much simpler technics usually suffice for the identification of hemoglobin.

Fautrez and Lambert did not believe cyanol was acting as a peroxidase stain in their hemoglobin-staining method. However, cyanol, color index no. 715, is closely related chemically to patent blue V, color index no. 712, and this chemical relationship suggested that it might be used in Lison's technic [2] as modified by Dunn.[3]

By thus substituting cyanol for patent blue V, a very satisfactory hemoglobin stain is obtained. This cyanol-peroxidase reaction has been found to be highly specific for hemoglobin (erythrocytes, hemoglobin-containing renal casts and globules) in paraffin sections of tissue fixed in 4 per cent solution of formaldehyde buffered to p_H 7.0. It has been tested on renal tissue of hemoglobinuric mice, rats, guinea pigs, dogs and monkeys. Hemoglobin stains dark blue to light bluish gray. The specificity and sensitivity of this cyanol hemoglobin stain is equal to that of patent blue V, in most instances. Cyanol is also useful on frozen sections and methylalcohol-fixed blood smears, but it is not recommended for the latter purpose unless the smear is very well fixed, as the blood film often becomes detached.

TECHNIC

The cyanol stock solution is prepared by dissolving 1 Gm. of cyanol [4] in 100 cc. of distilled water, then adding 10 Gm. of zinc powder, chemically pure and 2 cc. of glacial acetic acid. This mixture is brought to the boiling point. In a short time complete decolorization (loss of blue color) will occur. This solution is stable for several weeks. To prepare working reagent: Filter, just before use, 10 cc. of stock solution and add 2 cc. of glacial acetic acid and 1 cc. of commercial hydrogen peroxide (3 per cent).

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* From the Pathology Laboratory, National Institute of Health.

1. Fautrez, J., and Lambert, P. P.: Bull. d'histol. appliq. à la physiol. **14:** 29, 1937.

2. Lison, L.: Beitr. z. path. Anat. u. z. allg. Path. **101:**94, 1938.

3. Dunn, R. C.: A Simplified Stain for Hemoglobin Using Patent Blue, to be published.

4. The cyanol (color index 715) used was furnished by National Aniline Division, Allied Chemical & Dye Corporation.

To prepare safranin counterstain: Make a 1:1,000 safranin solution in 1 per cent acetic acid.

To stain sections: Bring sections to water; stain in freshly prepared working reagent three to five minutes; rinse in water; counterstain in the safranin solution about one minute; rinse in water; dehydrate with alcohols; clear in xylene; mount in clarite.

Results: Hemoglobin stains dark blue to bluish gray, the nuclei red and the cytoplasm light pink.

The total time required for staining is about ten minutes.

SUMMARY

The hemoglobin stain described may be used to identify hemoglobin in frozen or paraffin sections of tissue fixed in 4 per cent solution of formaldehyde buffered to p_H 7.0.

The deparaffinized, hydrated sections are stained in the cyanol working reagent about four minutes, washed, counterstained in acidified safranin about one minute, rinsed in water, dehydrated and mounted in clarite. The time required exclusive of hydration and dehydration is about ten minutes. Hemoglobin stains blue to bluish gray.

Notes and News

Appointments, Etc.—C. G. Tedeschi, Medfield State Hospital, Harding, Mass., has been appointed director of the laboratory at Framingham Union Hospital, Framingham, Mass.

Birmingham Medical College, Birmingham, Ala., has announced appointments as follows: in pathology, R. D. Baker professor, J. D. Bush associate professor, A. E. Casey and J. A. Cunningham assistant professors; in bacteriology and clinical pathology, R. McBurney professor and W. H. Riser associate professor of clinical pathology.

William C. White, Washington, D. C., has resigned as chairman of the committee on medical research of the National Tuberculosis Association, ending a service of more than twenty-five years. Under his chairmanship the committee made about fifty grants in support of research, amounting in all to some $600,000.

S. W. Lippincott, recently released from the Army Institute of Pathology, has been appointed professor of pathology and executive of the department of pathology at the new school of medicine of the University of Washington.

Death.—Simon Flexner, pathologist and bacteriologist, the first director of the Rockefeller Institute of Medical Research, died May 2, 83 years old.

Award.—O. T. Avery, of the Rockefeller Institute for Medical Research, New York, has been awarded the Kober Medal of Georgetown University School of Medicine, Washington, D. C., for his work on problems of pneumonia.

Committee on Growth of the National Research Council.—This committee, which acts for the American Cancer Society, will consider applications for grants in aid of cancer research to become effective July 1, 1947. Applications will be received until Sept. 15, 1946. Applications for fellowships in cancer research for the ensuing year will be received until Dec. 1, 1946. Application for grants for the current year will no longer be entertained.

To date the committee has recommended seventy-five research grants and fourteen fellowships. It will continue to recommend support of research in the basic sciences and in clinical investigative medicine broadly pertaining to problems of growth. The address is Committee on Growth, Division of Medical Sciences, National Research Council, 2101 Constitution Avenue, Washington 25, D. C.

Endowment for Surgical Research.—Theodore L. Case, retired Philadelphia surgeon, has given Temple University School of Medicine $1,000,000 to establish a surgical research foundation with special emphasis on cancer.

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